PREPARATION AND EVALUATION OF *MOMORDICA CHARANTIA* NANOPHYTOSOMES AND EFFICACY ON MDA-MB HUMAN BREAST CANCER CELL LINES

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MASTER OF PHARMACY

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By

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This is certify that the investigation described in this dissertation entitled **"PREPARATION AND EVALUATION OF *MOMORDICA CHARANTIA* NANOPHYTOSOMES AND EFFICACY ON MDA-MBHUMAN BREAST CANCER CELL LINES"** Submitted by Reg.No: 261610403 was carried out in the Department of Pharmaceutics, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626126, Which is affiliated to The Tamilnadu Dr.M.G.R.Medical University,Chennai, Under the Guidance of Dr.C.Sowmya, M.Pharm., Ph.D., Professor, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil.

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CONTENTS

INTRODUCTION

1. INTRODUCTION

1.1 CANCER

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. These contrast with benign tumors, which do not spread to other parts of the body. Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements. While these symptoms may indicate cancer, they may have other causes. Over 100 types of cancers affect humans.Tobacco use is the cause of about 22% of cancer deaths. Another 10% are due to obesity, poor diet, lack of physical activity, and excessive drinking of alcohol. Other factors include certain infections, exposure to ionizing radiation and environmental pollutants. In the developing world nearly 20% of cancers are due to infections such as hepatitis B, hepatitis C and human papilloma virus infection. These factors act, at least partly, by changing the genes of a cell. Typically many genetic changes are required before cancer develops. Approximately 5–10% of cancers are due to inherited genetic defects from a person's parents. Cancer can be detected by certain signs and symptoms or screening tests. It is then typically further investigated by medical imaging and confirmed by biopsy. Many cancers can be prevented by not smoking, maintaining a healthy weight, not drinking too much alcohol, eating plenty of vegetables, fruits and whole grains, vaccination against certain infectious diseases, not eating too much processed and red meat, and avoiding too much sunlight exposure. Early detection through screening is useful for cervical and colorectal cancer. The benefits of screening in breast cancer are controversial. Cancer is often treated with some combination of radiation therapy, surgery, chemotherapy, and targeted therapy. Pain and symptom management are an important part of care. Palliative care is particularly important in people with advanced disease[1].

1.1.1. Signs and Symptoms

When cancer begins, it produces no symptoms. Signs and symptoms appear as the mass grows or ulcerates. The findings that result depend on the cancer's type and location. Few symptoms are specific. Many frequently occur in individuals who have other conditions. Cancer is a "great imitator". Thus, it is common for people

diagnosed with cancer to have been treated for other diseases, which were hypothesized to be causing their symptoms. People may become anxious or depressed post-diagnosis. The risk of suicide in people with cancer is approximately double.

Local symptoms

Local symptoms may occur due to the mass of the tumor or its ulceration. For example, mass effects from lung cancer can block the bronchus resulting in cough or pneumonia; esophageal cancer can cause narrowing of the esophagus, making it difficult or painful to swallow; and colorectal cancer may lead to narrowing or blockages in the bowel, affecting bowel habits. Masses in breasts or testicles may produce observable lumps. Ulceration can cause bleeding that, if it occurs in the lung, will lead to coughing up blood, in the bowels to anaemia or rectal bleeding, in the bladder to blood in the urine and in the uterus to vaginal bleeding. Although localized pain may occur in advanced cancer, the initial swelling is usually painless. Some cancers can cause a build up of fluid within the chest or abdomen.

Systemic Symptoms

General symptoms occur due to effects that are not related to direct or metastatic spread. These may include: unintentional weight loss, fever, excessive fatigue and changes to the skin. Hodgkin disease, leukemia and cancers of the liver or kidney can cause a persistent fever. Some cancers may cause specific groups of systemic symptoms, termed paraneoplastic syndrome. Examples include the appearance of myasthenia gravis in thymoma and clubbing in lung cancer[2].

1.1.2. Causative Factors of Cancer

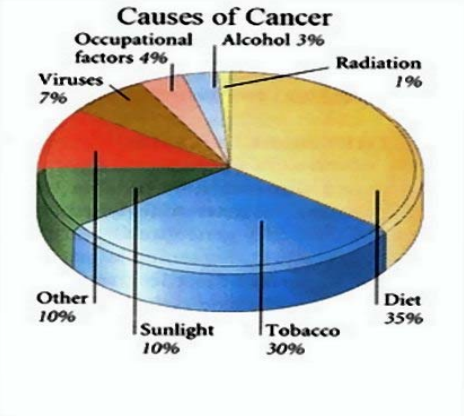


Figure 1.1: Causative factors of cancer

I. Chemicals

Particular substances have been linked to specific types of cancer. Tobacco smoking is associated with many forms of cancer, and causes 80% of lung cancer. Daily long-term vaping with a high voltage (5.0 V) electronic cigarette may generate formaldehyde-forming chemicals at a greater level than smoking, which was determined to be a lifetime cancer risk of approximately 5 to 15 times greater than smoking. Many mutagens are also carcinogens, but some carcinogens are not mutagens. Alcohol is an example of a chemical carcinogen that is not a mutagen. In Western Europe 10% of cancers in males and 3% of cancers in females are attributed to alcohol. Decades of research has demonstrated the link between tobacco use and cancer in the lung, larynx, head, neck, stomach, bladder, kidney, esophagus and pancreas. Tobacco smoke contains over fifty known carcinogens, including nitrosamines and polycyclic aromatic hydrocarbons. Tobacco is responsible for about one in three of all cancer deaths in the developed world, and about one in five worldwide[3].

II. Diet and Exercise

Diet, physical inactivity, and obesity are related to approximately 30–35% of cancer deaths. In the United States excess body weight is associated with the development of many types of cancer and is a factor in 14–20% of all cancer deaths. Physical inactivity is believed to contribute to cancer risk not only through its effect on body weight but also through negative effects on immune system and endocrine system. More than half of the effect from diet is due to over nutrition rather than from eating too little healthy foods. Diets that are low in vegetables, fruits and whole grains, and high in processed or red meats are linked with a number of cancers. A high-salt diet is linked to gastric cancer, aflatoxin B1, a frequent food contaminate, with liver cancer, and Betel nut chewing with oral cancer. This may partly explain differences in cancer incidence in different countries. For example,gastric cancer is more common in Japan due to its high-salt diet and colon cancer is more common in the United States. Immigrants develop the risk of their new country, often within one generation, suggesting a substantial link between diet and cancer[4].

III. Infection

Worldwide approximately 18% of cancer deaths are related to infectious diseases. Viruses are the usual infectious agents that cause cancer but bacteria and parasites may also have an effect. A virus that can cause cancer is called an *onco virus*. These include human papilloma virus(cervical carcinoma), Epstein–Barr virus (B-cell lympho proliferative disease and nasopharyngeal carcinoma), Kaposi's sarcoma herpes virus (Kaposi's sarcoma and primary effusion lymphomas), hepatitis B and hepatitis C viruses (hepato cellular carcinoma), and Human T-cell leukemia virus-1 (T-cell leukemias). Bacterial infection may also increase the risk of cancer, as seen in Helicobacter pylori-induced gastric carcinoma. Parasitic infections strongly associated with cancer include *Schistosoma haematobium* (squamous cell carcinoma of the bladder) and the liver flukes, *Opisthorchis viverrini* and *Clonorchis sinensis*(cholangiocarcinoma)[5].

IV. Radiation

Up to 10% of invasive cancers are related to radiation exposure, including both ionizing radiation and non-ionizing ultraviolet radiation. Additionally, the vast majority of non-invasive cancers are non-melanoma skin cancers caused by non-ionizing ultraviolet radiation. Sources of ionizing radiation include medical imaging, and radon gas. Some people, such as those with nevoid basal cell carcinoma syndrome or retinoblastoma, are more susceptible than average to developing cancer from radiation exposure. Children and adolescents are twice as likely to develop radiation-induced leukemia as adults; radiation exposure before birth has ten times the effect. Ionizing radiation is not a particularly strong mutagen. Residential exposure to radon gas, for example, has similar cancer risks as passive smoking. Low-dose exposures, such as living near a nuclear power plant, are generally believed to have no or very little effect on cancer development. Radiation is a more potent source of cancer when it is combined with other cancer-causing agents, such as radon gas exposure plus smoking tobacco. Prolonged exposure to ultraviolet radiation from the sun can lead to melanoma and other skin malignancies. Clear evidence establishes ultraviolet radiation, especially the non-ionizing medium wave UVB, as the cause of most non-melanoma skin cancers,

which are the most common forms of cancer in the world. Non-ionizing radio frequency radiation from mobile phones, electric power transmission, and other similar sources have been described as a possible carcinogen by the World Health Organization's International Agency for Research on Cancer[6].

V. Hereditary Causes

The vast majority of cancers are non-hereditary ("sporadic cancers"). Hereditary cancers are primarily caused by an inherited genetic defect. Less than 0.3% of the population are carriers of a genetic mutation which has a large effect on cancer risk and these cause less than 3–10% of all cancer. Some of these syndromes include: certain inherited mutations in the genes *BRCA1* and *BRCA2* with a more than 75% risk of breast cancer and ovarian cancer, and hereditary non polypos is colorectal cancer (HNPCC or Lynch syndrome) which is present in about 3% of people with colorectal cancer, among others[7].

VI. Physical Agents

Some substances cause cancer primarily through their physical, rather than chemical, effects on cells. A prominent example of this is prolonged exposure to asbestos, naturally occurring mineral fibers which are a major cause of mesothelioma, which is a cancer of theserous membrane, usually the serous membrane surrounding the lungs. Other substances in this category, including both naturally occurring and synthetic asbestos-like fibers such aswollastonite, attapulgite, glass wool, and rock wool, are believed to have similar effects. Non-fibrous particulate materials that cause cancer include powdered metallic cobalt andnickel, and crystalline silica (quartz, cristobalite, and tridymite). Usually, physical carcinogens must get inside the body (such as through inhaling tiny pieces) and require years of exposure to develop cancer[8].

VII. Hormones

Some hormones play a role in the development of cancer by promoting cell proliferation. Insulin-like growth factors and their binding proteins play a key role in cancer cell proliferation, differentiation and apoptosis, suggesting possible involvement in carcinogenesis. Hormones are important agents in sex-related

cancers such as cancer of the breast, endometrium, prostate, ovary, and testis, and also of thyroid cancer and bone cancer. For example, the daughters of women who have breast cancer have significantly higher levels of estrogen and progesterone than the daughters of women without breast cancer. These higher hormone levels may explain why these women have higher risk of breast cancer, even in the absence of a breast-cancer gene[9].

VIII. Others

Excepting the rare transmissions that occur with pregnancies and only a marginal few organ donors, cancer is generally not a transmissible disease. The main reason for this is tissue graft rejection caused by MHC incompatibility. In humans and other vertebrates, the immune system uses MHC antigens to differentiate between "self" and "non-self" cells because these antigens are different from person to person. When non-self antigens are encountered, the immune system reacts against the appropriate cell. Such reactions may protect against tumor cell engraftment by eliminating implanted cells. In the United States, approximately 3,500 pregnant women have a malignancy annually, and trans placental transmission of acute leukemia, lymphoma, melanoma and carcinoma from mother to fetus has been observed. The development of donor-derived tumors from organ transplants is exceedingly rare. The main cause of organ transplant associated tumors seems to be malignant melanoma, that was undetected at the time of organ harvest. Job stress does not appear to be a significant factor at least in lung, colorectal, breast and prostate cancers[10].

1.1.3.Classification of Cancer

A. Carcinoma

Cancer that begins in the skin or in tissues that line or cover internal organs likes Skin cancer, Lung cancer, Colon cancer, pancreatic cancer, ovarian cancer. Epithelial carcinoma, Squamous carcinoma and Basal cell carcinoma, Melanoma, Papilloma, and Adenoma.

B. Sarcoma

Cancer that begins in bone, cartilage, fat, muscle, blood vessels, or other connective tissues or supportive tissues is also called as bone cancer. Osteo sarcoma, Synovial sarcoma, Lipo sarcoma, Angino sarcoma, Rhabdo sarcoma, and Fibro sarcoma.

C. Leukemia

Cancer that starts in blood forming tissue such as the bone marrow and causes large numbers of abnormal blood cells to be produced and enter the blood is also called as leukemia. Lymphoblastic leukemia, Myelogenous leukemia, T-cell leukemia, and Hairy-cell leukemia.

D. Lymphoma and myeloma

cancer that begins in the cells of the immune system is called as lymphoma. T-cell lymphoma, B-cell lymphoma, Hodgkin lymphoma, Non-Hodgkin lymphoma and Lympho proliferative lymphoma.

E. Central nervous system cancers

Cancer that begins in the tissues of the brain and spinal cord is called as brain and spinal cord cancer. Glioma, Meningiomas, Pituitary adenoma,Vestibular schwannoma, Primary CNS lymphoma, Primitive neuroectodermal cancer[11].

1.1.4. Difference Between Normal Cells and Cancer Cells

A. Normal Cells

Normal cells carry characterstics that are essential for normal body functions. These cells have different in shapes and sizes but are uniform depending on what type they are. Human cells are eukaryotic because they contain true nucleus that contains genetic information DNA and RNA. These genes are responsible for all cellular activities and functioning. Healthy cells Divide in an orderly manner to produce more cells only when the body needs them. They follow a life cycle which includes mitosis and meiosis, and cell death-apoptosis.

B. Cancer Cells

There are two distinct features that cancer cells have, cell growth not regulated by external signals and the capacity to invade tissue and colonize distant sites. The uncontrolled growth of abnormal cells is a property of all neoplasm. Neoplasm can either be benign or malignant[12].

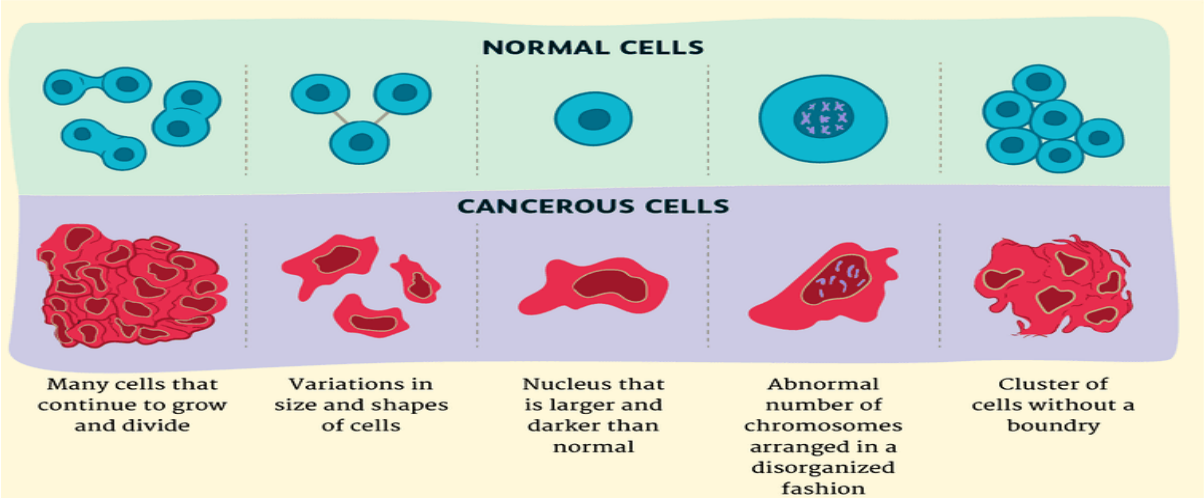


Figure 1.2: Difference between normal cells and cancer cells

Table 1.1: Difference between normal cells and cancer cells

CELL CHARACTERISTICS	NORMAL CELLS	CANCER CELLS
MORPHOLOGY	Normal cells have uniform shapes and sizes.	Cancer cells have a large variety of sizes and shapes. The nucleuses have irregular structure and have relatively small cytoplasm.

REPRODUCTION AND CELL DEATH	• Cells stop dividing when too much of its kind are present. • These cells grow and divide in a controlled manner and follow a predictable life cycle. • Normal cells undergo the process of apoptosis – self destruction if they detect abnormalities and damage in their organelles.	Cancer cells don't stop growing resulting to appearance of a tumor (a cluster of mutant cells)
COMMUNICATION	Normal cells communicate with each other for proper functioning.	Cancer cells do not communicate with each other
ADHESION AND INVASION	These cells have external membranes that allow them to bond with other cells.	These cells have the ability to invade or spread to other parts of the body by travelling through the blood stream or the lymphatic system – metastasis.
SPECIALIZATION	Normal cells start out as immature cells and mature with certain specialized functions.	Cancer cells do not mature, and undergo apoptosis. Instead these cells become immature overtime.Cancer cells are primitive and they don't have specialized functions.

SIGNAL RECOGNITION	Normal cells recognize signals. They know when there are enough new cells and stops dividing.	Cancer cells don't recognize signals. Hence these cells erratically reproduce mutated cells.

1.1.5. Development of Cancer

Cancerous cells develop from healthy cells in a complex process called malignant transformation.

Initiation

The initial phase in tumor advancement is start, in which an adjustment in the cell's genetic material takes action to wind up plainly destructive. The adjustment in the cell's genetic material may happen suddenly or be expedited by an operator that causes tumor (a cancer-causing agent). Cancer-causing agents incorporate numerous chemicals, tobacco, infections, radiation, and sunlight. In any case, not all cells are similarly vulnerable to cancer-causing agents. A genetic defect in a cell may make it more helpless. Even chronic physical irritation may make a cell more susceptible to carcinogens.

Promotion

The second and last step in the improvement of malignancy is promotion. Operators that reason promotion, or promoters, might be substances in nature or even a few medications, for example, sex hormones (for instance, testosterone taken to enhance sex drive and vitality in more established men). Not at all like cancer-causing agents, promoters don't cause malignancy without anyone else. Rather, promoters permit a cell that has experienced starts to wind up plainly malignant. Advancement has no impact on cells that have not experienced start. In this manner, a few components, frequently the blend of a powerless cell and a cancer-causing agent, are expected to cause disease. A few cancer-causing agents are adequately effective to have the capacity to cause tumor without the requirement for advancement. For instance, ionizing radiation (which is utilized as a part of x-beams

and is created in atomic power plants and nuclear bomb blasts) can cause different tumors, especially sarcomas, leukemia, thyroid disease, and bosom malignancy.

Spreading

Tumor can grow directly into surrounding tissue or spread to tissues or organs, adjacent or far off. Growth can spread through the lymphatic system. This kind of spread is run of the mill of carcinomas. For instance, bosom tumor more often than not spreads initially to the adjacent lymph hubs in the armpit, and just later does it spread too far off locales. Tumor can also spread by means of the circulatory system. This sort of spread is regular of sarcomas[13].

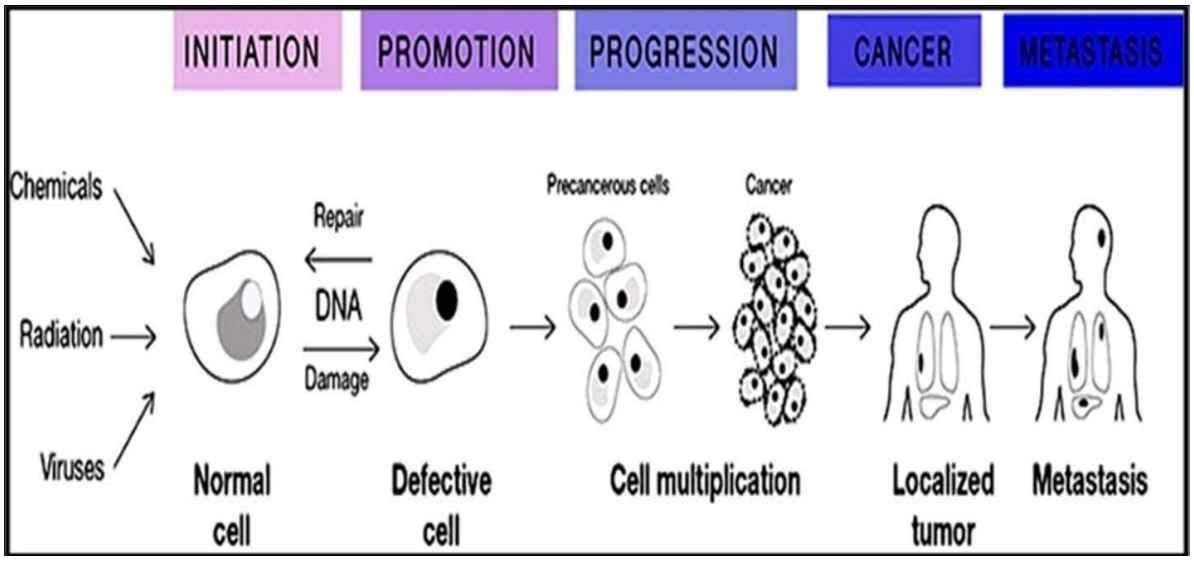


Figure 1.3: The progress of metastasis *(Courtesy by Waheed Roomi et al., 2016, Cellular Medicine & Natural Health, June 15, 2016)*

1.1.6. Cancer Treatment

I.Chemotheraphy

Chemotheraphy is the treatment of tumor with at least one cytotoxic hostile to neoplastic medications (chemotherapeutic specialists) as a major aspect of an institutionalized regimen. The term incorporates an assortment of medications, which are partitioned into general classes, for example, alkylation specialists and anti metabolites. Conventional chemotherapeutic specialists act by executing cells that separation quickly, a basic property of most growth cells. Directed treatment is a type

of chemotherapy that objectives particular sub-atomic contrasts amongst disease and ordinary cells[14]. The main focused on treatments hindered the estrogen receptor molecule, restraining the development of bosom tumor. Another common instance is the class of Bcr-Abl inhibitors, which are used to treat continual myelogenous leukemia (CML).Currently, centered therapies exist for breast cancer, multiple myeloma, lymphoma, prostate cancer, melanoma and different cancers. The efficacy of chemotherapy depends on the type of cancer and the stage. In combination with surgery, chemotherapy has proven useful in cancer types which include breast cancer, colorectal cancer, pancreatic cancer, estrogenic sarcoma, testicular cancer, ovarian cancer and positive lung cancers. Chemotherapy is healing for some cancers, such as some leukemia's, ineffective in some brain tumors. and unnecessary in others, such as most non-melanoma pores and skin cancers. The effectiveness of chemotherapy is regularly constrained by way of its toxicity to other tissues in the body. Even when chemotherapy does no longer provide a everlasting cure, it may additionally be beneficial to limit signs such as ache or to limit the dimension of an inoperable tumor in the hope that surgical operation will end up feasible in the future[15].

II.Radiation therapy

Radiation therapy entails the use of ionizing radiation in an strive to either remedy or enhance symptoms. It works via detrimental the DNA of cancerous tissue, killing it. To spare normal tissues (such as pores and skin or organs, which radiation ought to ignore via to treat the tumor), fashioned radiation beams are aimed from a couple of publicity angles to intersect at the tumor, imparting a lots larger dose there than in the surrounding, wholesome tissue. As with chemotherapy, cancers differ in their response to radiation therapy. Radiation remedy is used in about half of cases. The radiation can be both from inner sources (brachytherapy) or exterior sources[16]. The radiation is most usually low power x-rays for treating pores and skin cancers, while greater energy x-rays are used for cancers within the body. Radiation is typically used in addition to surgical treatment and or chemotherapy. For positive types of cancer, such as early head and neck cancer, it may additionally be used alone. For painful bone metastasis, it has been determined to be nice in about 70% of patients[17].

III.Surgery

Surgery is the fundamental technique of remedy for most isolated, strong cancers and may play a function in palliation and prolongation of survival. It is typically an important phase of definitive prognosis and staging of tumours, as biopsies are typically required. In localized cancer, surgical operation generally attempts to dispose of the whole mass along with, in positive cases, the lymph nodes in the area. For some types of cancer this is sufficient to dispose of the cancer.

IV.Palliative-care

Palliative care refers to cure that tries to help the affected person experience higher and can also be mixed with a try to treat the cancer. Palliative care includes motion to limit physical, emotional, spiritual and psycho-social distress. Unlike therapy that is aimed at immediately killing most cancers cells, the major purpose of palliative care is to enhance fine of life. People at all degrees of cancer cure normally acquire some kind of palliative care. In some cases, medical specialty expert businesses advise that sufferers and medical practitioner reply to cancer only with palliative care. This applies to sufferers who display low overall performance status, implying restrained potential to care for themselves received no gain from prior evidence-based treatments are not eligible to take part in any excellent clinical trial no robust proof implies that treatment would be effective. Palliative care may also be harassed with hospice and therefore only indicated when human beings method stops of life. Like hospice care, palliative care tries to help the affected individual cope with their immediately desires and to extend comfort. Unlike hospice care, palliative care does now not require human beings to supply up cure aimed at the cancer.

Multiple national scientific pointers suggest early palliative care for patients whose cancer has produced distressing signs and symptoms and signs or who need assist coping with their illness. In suffers first recognized with metastatic disease, palliative care can also be immediately indicated. Palliative care is indicated for patients with a prognosis of much less than 12months of lifestyles even given aggressive treatment[19].

V.Immunotherapy

A range of remedies using immunotherapy, stimulating or supporting the immune system to battle cancer, have come into use given that 1997. Approaches encompass antibodies, checkpoint therapy and adoptive cellophane transfer[20].

VI.Laser-therapy

Laser remedy uses high-intensity mild to deal with cancer via shrinking or destroying tumors or precancerous growths. Lasers are most typically used to deal with superficial cancers that are on the floor of the body or the lining of inner organs. It is used to treat basal cellophane pores and skin cancer and the very early tiers of others like cervical, penile, vaginal, vulgar, and non-small mobile lung cancer. It is frequently combined with other treatments, such as surgery chemotherapy, or radiation therapy. Laser-induced interstitial thermotherapy (LITT), or interstitial laser photocoagulation, makes use of lasers to treat some cancers the usage of hyperthermia, which makes use of warmth to reduce tumors with the aid of negative or killing most cancers cells. Laser are more particular than surgery and motive much less damage, pain, bleeding, swelling, and scarring. A downside is surgeons have to have specialized training. It may additionally be extra steeply-priced than different treatments[21].

VII. Alternative Medicine

Complementary and choice most cancers treatments are a numerous group of therapies, practices and products that are now not part of conventional medicine."Complementary medicine" refers to strategies and materials used alongside with traditional medicine, whilst "alternative medicine" refers to compounds used instead of traditional medicine. Most complementary and choice medicines for cancer have no longer been studied or examined using conventional methods such as scientific trials. Some choice remedies have been investigated and shown to be ineffective however nonetheless proceed to be marketed and promoted[22,23].

1.2.BREAST CANCER

The breast is made up of different tissue, ranging from very fatty tissue to very dense tissue. Within this tissue is a network of lobes. Each lobe is made up of tiny, tube-like

structures called lobules that contain milk glands. Tiny ducts connect the glands, lobules, and lobes, carrying milk from the lobes to the nipple. The nipple is located in the middle of the areola, which is the darker area that surrounds the nipple. Blood and lymph vessels also run throughout the breast. Blood nourishes the cells. The lymph system drains bodily waste products. The lymph vessels connect to lymph nodes, the tiny, bean-shaped organs that help fight infection. Cancer begins when healthy cells in the breast change and grow out of control, forming a mass or sheet of cells called a tumor. A tumor can be cancerous or benign. A cancerous tumor is malignant, meaning it can grow and spread to other parts of the body. A benign tumor means the tumor can grow but will not spread. Breast cancer spreads when the cancer grows into other parts of the body or when breast cancer cells move to other parts of the body through the blood vessels and/or lymph vessels. This is called metastasis. This guide covers early-stage and locally advanced breast cancer, which includes stages I, II, and III. The stage of breast cancer describes where the cancer is located, how much the cancer has grown, and if or where it has spread. Although breast cancer most commonly spreads to nearby lymph nodes, it can also spread further through the body to areas such as the bones, lungs, liver, and brain. This is called metastatic or stage IV breast cancer[24].

Types of breast cancer

Breast cancer can be invasive or noninvasive. Invasive breast cancer is cancer that spreads into surrounding tissues. Noninvasive breast cancer does not go beyond the milk ducts or lobules in the breast. Most breast cancers start in the ducts or lobes and are called ductal carcinoma or lobular carcinoma:

Ductal carcinoma: These cancers starts in the cells lining the milk ducts and make up the majority of breast cancers.

Ductal carcinoma in situ (DCIS): This is cancer that is located only in the duct.

Invasive or infiltrating ductal carcinoma: This is cancer that has spread outside of the duct.

Lobular carcinoma: This is cancer that starts in the lobules.

Lobular carcinoma in situ (LCIS): This cancer is located only in the lobules. LCIS is not considered cancer. However, LCIS is a risk factor for developing invasive breast cancer in both breasts.

Signs and Symptoms

A lump that feels like a hard knot or a thickening in the breast or under the arm. It is important to feel the same area in the other breast to make sure the change is not a part of healthy breast tissue in that area.

- Change in the size or shape of the breast
- Nipple discharge that occurs suddenly, is bloody, or occurs in only 1 breast
- Physical changes, such as a nipple turned inward or a sore in the nipple area
- Skin irritation or changes, such as puckering, dimpling, scaliness, or new creases
- Warm, red, swollen breasts with or without a rash with dimpling resembling the skin of an orange, called “peau d'orange”
- Pain in the breast, particularly breast pain that does not go away. Pain is not usually a symptom of breast cancer, but it should be reported to a doctor.

Causes

After puberty, a woman's breast consists of fat, connective tissue, and thousands of lobules, tiny glands that produce milk for breast-feeding. Tiny tubes, or ducts, carry the milk toward the nipple. In cancer, the body's cells multiply uncontrollably. It is the excessive cell growth that causes cancer. Breast cancer usually starts in the inner lining of milk ducts or the lobules that supply them with milk. From there, it can spread to other parts of the body[25].

Stages

Cancer is staged according to the size of the tumor and whether it has spread to lymph nodes or other parts of the body.

There are different ways of staging breast cancer. One way is from stage 0 to 4, but these may be broken down into smaller stages.

Stage 0: Known as ductal carcinoma in situ (DCIS), the cells are limited to within a duct and have not invaded surrounding tissues.

Stage 1: At the beginning of this stage, the tumor is up to 2 centimeters (cm) across and it has not affected any lymph nodes.

Stage 2: The tumor is 2 cm across and it has started to spread to nearby nodes.

Stage 3: The tumor is up to 5 cm across and it may have spread to some lymph nodes.

Stage 4: The cancer has spread to distant organs, especially the bones, liver, brain, or lungs[26].

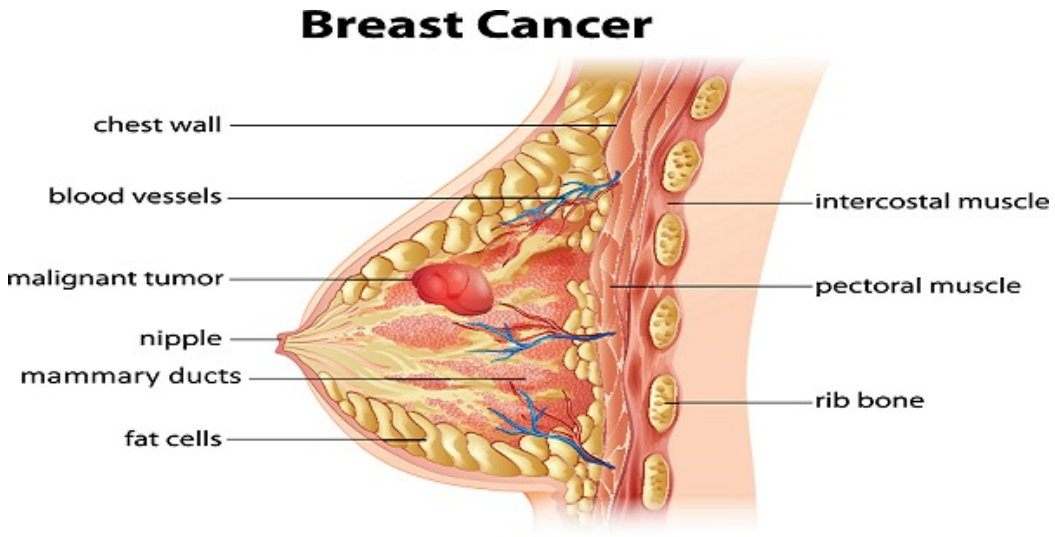


Figure1.4: Anatomy of the breast cancer

1.3 ANTI CANCER PLANTS

Medicinal plants play a significant role as therapeutic aid in health system in all over the world. Therefore from ancient period to modern era herbal drugs have been used to cure several diseases. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body. These medicinal plants constitute an important group of non wood forest products and

represent a vast potential source for anti cancer compounds. Approximately 80% of the world population uses plant as a source of medicine for health care. India has been one of the pioneers in the devolpment and practice of well documented indigenous system of medicine, particularly Ayurvedha, Siddha and Unani System, all these system of medicine are gaining world wide popularity. According to WHO essential medicine list contain 252 drugs out of which 11% is exclusively of plant origin. Consequently, there has been great demand for these plants but regrettably only a few medicinal plants are being cultivated on a commercial scale with a majority still being collected from the wild. Due of over exploitation of plants have become endangered. For survival there is a need to conserve the wild population of plant in the natural or in wild condition to meet the commercial needs through cultivation. In the present scenario herbal medicine are in evolutionary process for developing new medicines therefore pharmaceutical companies are involved in research on plant materials for their potential medicinal values as the demand for herbal products is growing exponentially due to its fewer side effects as compared to other system of medicines. According to WHO cancer is the second most frequent cause of death in developed countries. Many cancer patients use complementary and alternative medicine treatments. A wide range of knowledge regarding pharmacological research has considerably in improved the quality of herbal drugs in cancer treatment. Among several treatments Homeopathy is one of the most popular complementary and alternative medicine modalities for cancer patients but due to the lack of knowledge about its utilization, is major factor for threatening the large scale for cultivation of plants[27]. Some important well known phytochemical used for the treatment of cancer are listed below as.

Table 1.2: List of plants having anti cancer activity[28-35]

S.NO	PLANTS NAME	FAMILY	ACTIVE CONSTITUENTS
1.	*Actinidia chinensis*	*Actinidiaceae*	Polysaccharide
2	*Aegle marmelos*	*Rutaceae*	Lupeo
3	*Agapanthus africanus*	*Agapanthaceae*	Isoliquiritigenin
4	*Aglalia sylvestre*	*Meliaceae*	Silvesterol
5	*Ailanthus altissima*	*Simaraubaceae*	Ailnthone

6	*Allium cepa*	*Liliaceae*	Allicin, Alliin, Diallylsulphide, Quarcetin, Vitamin.C and E
7	*Allium sataivum*	*Liliaceae*	Allicin,Alliin,Diallylsulphide Alliinase,S-Allylcysteine, Di allyl trisulphide
8	*Aloe barbadensis*	*Liliaceae*	Aloe-emodin, Emodin,Aloin,Acemanan
9	*Alpinia galanga willd*	*Zingiberaceae*	Acetoxy chavicol acetate, Pinocembrin,Galangin
10	*Ananas comosus*	*Bromeliaceae*	Ananas bromelanin
11	*Andrographis paniculata*	*Acanthaceae*	Andrographolide
12	*Angelica sinensis*	*Umbeliferae*	Angelica
13	*Annona species*	*Annonaceae*	Acetogenins
14	*Aphanamixis polystachya*	*Meliaceae*	Amooranin
15	*Apium graveolens*	*Umbeliferae*	Apigenin
16	*Arctium lappa*	*Compositae*	Arctigenin
17	*Astragalus membranaceus*	*Papilionaceae*	Swainsonine
18	*Azadirecta indica*	*Meliaceae*	Liminoids,Limbolide
19	*Bauhinia variegate*	*Cesalpiniaceae*	Cyaniding glucoside, malvidin peonidin glucoside and kaempferol
20	*Berberis vulgaris linn*	*Berberidaceae*	Berberine
21	*Betula utilis*	*Betulaceae*	Betulin
22	*Bleckeria vitensis*	*Apocyanaceae*	Ellipticine
23	*Brucea antidysenteria*	*Simaraubaceae*	Bruceantin
24	*Bursera microphylla*	*Burseraceae*	Burseran
25	*Camellia sinensis*	*Theaceae*	Epigallocatechin gallate
26	*Campotheca Acuminate*	*Nyssaceae*	Campothecin
27	*Catharanthus roseus*	*Apocyanaceae*	Vinblastin,Vincristine, Alstonin,Ajmalicine and

			Reserpine
28	*Centaurea montata*	*Asteraceae*	Montamine
29	*Centaurea schischkinii*	*Asteraceae*	Schischkinnin
30	*Cephalotaxus harringtonia*	*Cephalotaxaceae*	Homoharringtonine
31	*Chlorella pyrenoidosa*	*Oocystaceae*	Lysine
32	*Cleistanthus collinus*	*Euphorbiaceae*	Cleistanthin,collinusin
33	*Colchicum luteum*	*Liliaceae*	Colchicine
34	*Combretum caffrum*	*Combretaceae*	Combrestatins
35	*Croton lechleri*	*Euphorbiaceae*	Tapsine
36	*Curcuma longa linn*	*Zinziberaceae*	Tumerone,Curcumine
37	*Daphne mezereum*	*Thymelaeaceae*	Mezerein
38	*Diphylleia grayi*	*Berberidaceae*	Diphyllin
39	*Dysoxylum binectariferum*	*Meliaceae*	Rohitukin
40	*Echinacea angustifolia*	*Asteraceae*	Arabinogalactan, Jucogalactoxyloglucans
41	*Emblica officinalis*	*Euphorbiaceae*	Ellagic acid,Gallic acid,Quercetin, Emblicannins A and B
42	*Erythroxylum pervillei*	*Erythroxylaceae*	Pervilleine
43	*Euphorbia semiperfoliata*	*Euphorbiaceae*	Jatrophane
44	*Fagopyrum esculentum*	*Polygonaceae*	Amygdalin,rutin
45	*Fragaria vesca linn*	*Rosaceae*	Borneol,Ellagic acid
46	*Fritillaria thunbergii*	*Liliaceae*	Zhebeinone
47	*Ginkgo biloba*	*Ginkoaceae*	Ginkgolide-B,A,C and J
48	*Glysine max*	*Leguminosae*	Zinc,Selenium,Vitamins(A, B1,B2,B12,C,D,E,K), Amino acid,Isoflavones,Protease inhibitors,Saponins and Phytosterol
49	*Glycyrrhiza glabra*	*Leguminosae*	Glycyrrhizin
50	*Gossypium barbadensis*	*Malvaceae*	Gossypol
51	*Gunnera perpensa*	*Gunneraceae*	2-methyl-6(3-methyl 2-butenyl)benzo 1-4 quinone

52	*Gyrophora esculenta*	*Umbellicariaceae*	Poly saccharides β-glucans,α-glucans and Galactomannans
53	*Hypericum perforatum*	*Clusiaceae*	Hypericin
54	*Indigofera tinctoria*	*Leguminosae*	Indirubins
55	*Justicia procumbens*	*Acanthaceae*	Justicidin A,B
56	*Lantana camara*	*Verbbenaceae*	Verbascoside
57	*Larrea tridentate*	*Zygophyllaceae*	Terameprocol
58	*Lentinus edodes*	*Agaricaceae*	Lentinan
59	*Linium album*	*Linaceae*	Podophyllotoxin
60	*Linum usitatissimum*	*Linaceae*	Cynogenetic glycoside,Lignans
61	*Lonicera japonica*	*Caprifoliaceae*	Luteolin
62	*Zingiber officinale*	*Zingiberaceae*	Curcumin,GingerenoneA, Gingeols, Shogaols,Zingerone
63	*Menthe species*	*Labiateae*	Monoterpene ketones
64	*Morinda citrifolia linn*	*Rubiaceae*	Damnacanthal,Rubiiadin-methyl ether,Alizarin,Morindone and Anthragallol-2,3-dimethyl ether,Damnacanthol
65	*Nigella sativa linn*	*Ranunculaceae*	Thymoquinone, Dithymoquinone
66	*Ochrosia elliptica*	*Apocynaceae*	Ellipticine and 9-methoxy ellipticine are Pyridocarbazole,Alkaloids
67	*Ocimum sanctum linn*	*Lamiaceae*	Eugenol, Orientinand vicenin
68	*Oldenlandia diffusa roxb*	*Rubiaceae*	Ursolic acid
69	*Panax ginseng*	*Aralaceae*	Ginsenosides,Panaxosides
70	*Pestemon deustus*	*Serophulariaceae*	Liriodendrin
71	*Phaleria macrocarpa*	*Thymelaeaceae*	Pinoresinol,Laricinesinol
72	*Picrorrhizia kurroa*	*Scrophulariaceae*	Picrorrhizia,Picrosides1,2,3 and Kutkoside
73	*Podophyllum emodii*	*Berberidaceae*	Ephipodophyllotoxin

74	*Podophyllum hexandrum*	*Berberidaceae*	Podophyllin,Astragalin
75	*Polygonum cuspidatum*	*Polygonaceae*	Resveratrol
76	*Prunella vulgaris linn*	*Labiatae*	Ursolic acid,Oleanolic acid
77	*Psoralea corylifolia linn*	*Fabaceae*	Bavachinin,Psoralidin, Psoralen
78	*Pteris multifida*	*Pteridaceae*	Pterokaurane
79	*Pygeum africanum*	*Rosaceae*	Amygdalin
80	*Rubia cordifolia linn*	*Rubiaceae*	Rubidianin,Rubiadin rosemary acids,Purpurine,Alizarin, Xanthopurpurin
81	*Saussurea lappa C.B Clarke*	*Compositae*	Cynaropicrin, Costunolide dehydrocostuslactone, Shikokio
82	*Solanum nigrum linn*	*Solanaceae*	Solamargine,Solasonine, Solanin,Quercetin
83	*Taxus brevifolia*	*Taxaceae*	Taxanes,Taxol,Cepholomann ine
84	*Tinospora cardifolia*	*Menispermaceae*	Berberine,Tinosporin,Giloin, Gilonin
85	*Viscum album*	*Viscaceae*	Lectin,Proprionyl choline,Lupeol,Viscotoxin, Digallic acid
86	*Vitex rotundifolia*	*Verbenaceae*	Korea casticin

1.3.1. Advantages of Anti Cancer Plants

- Good immunomodulatory.
- They promote host resistant against infection by stimulating both specific and non-specific immunity.
- Benefit of using plant derive product over synthetic medicine have increased the importance of medicinal plants in the healthcare.
- Many plants show in cancer treatment by inhibiting cancer activating enzymes.

- Stimulating DNA repair mechanism.
- Induce anti-oxidant action.
- To promote protective enzymes production.
- Plant derived compounds are more tolerated and non toxic to the normal human cells[36].

1.3.2. Disadvantages of Anti Cancer Plants

- The plant or plant materials are orally injected, it causes poor absorption and decreased bioavailability.

1.4. PHYTOSOMES

Phytosomes are said to be containing natural herbal formulations. Most of the Plants are having medicinal properties due to the presence of many active constituents which are mainly the secondary metabolites like flavonoids, terpinoids, tannins, glycosides, alkaloids etc. The active constituents present in the plants are mostly hydrophilic in nature. The therapeutic efficacy of herbal extracts are quickly destroyed by the enzymes present in the intestinal gut. Hence, advanced researches are done for the specific site delivery of these plant derived products[37]. The term “phyto” means plant and “some” means cell like[38]. It is also called as herbosomes. This is an advanced methodology, where extract of the plant or the hydrophilic phytoconstituents are mixed with phospholipids to produce more lipid stable molecular complexes, thereby it enhances the absorption and bioavailability of phytoconstituents[39,40,41]. Phospholipids are naturally used as an aid for digestion and act as carriers for both fat soluble and water soluble nutrients[42,43,44]. Phytosomes can easily cross the cell membranes and also stratum corneum layer of the skin[45,46]In the last century numerous research have been performed on a lot of plant extracts to know their biological importance and their use in medicinal field. Phytosomes have better ability to penetrate into the membrane of the cell and from there it enter into the cell and finally reaching the systemic circulation[47,48].

1.4.1. Phytosome Technology and its Advantages

Hydrophilic phytoconstituents has the ability to bind with phospholipids. A specified amount of phospholipid (phosphatidylcholine) react with the herbal extract in a non-

polar solvent. The phospholipid (phosphatidylcholine) used in this formulation was obtained from soybean with both lipophilic (phosphotidyl part) and hydroplilic (choline) portions. The body portion has choline group which is hydrophilic and the tail portion has phosphotidyl group which is lipophilic in nature, thereby the hydrophilic group is encoded within the lipophilic group to form a stable complex, phytosomes are formed[49,50]. The bonds formed are chemical in nature, which in additional provides better stability for the drug molecule in complex with wide range of advantages **(Table 1.3)**.

Phosphatidyl choline used in this formulation has dual function, it act as a carrier for drug moiety with nutritional value[51,52].

Table 1.3: advantages of phytosomes

S.No.	ADVANTAGES OF PHYTOSOMES[53-56]
1.	Hydrophilic herbal extracts absorption is enhanced and has a better therapeutic effect.
2.	Phytosomes deliver the drug at specific site, so low dose is required to produce therapeutic effect.
3.	Phytosomes are easy to develop and has more stability than any other herbal formulations.
4.	The carrier used in this formulation (phosphotidylcholine)has an advantage that it is eco-friendly with nutritional value.
5.	Drug entrapment capacity of phytosomes is high than compared to any other herbal formulations.
6.	No complex techniques are not required in the production of phytosomes and hence low cost is required for its production.
7.	Phytosomal formulations are easily penetrate through the layer of the skin. Hence it can use for transdermal delivery.

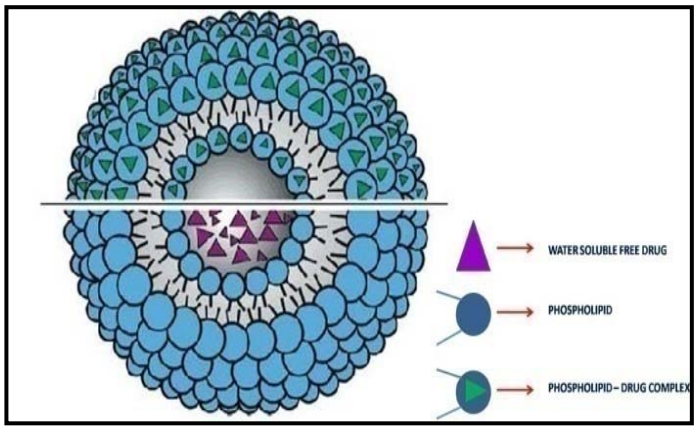


Figure 1.5(A & B): The difference between phytosome and liposome.

1.4.2. Preparation of Nanophytosomes

Though there was not enough data available throughout the phytosome research, authors tried maximum to provide all inputs for the preparation of phytosomes. The method for the preparation of phytosomes are as follows: In the first step, phospholipids are obtained from either natural or synthetic sources are to be dissolved in a organic solvent such as acetone or dioxane. To the solution of phospholipids, herbal extract is added with constant stirring. Then the solution is allowed to evaporate on a spray dryer.The ratio between the portions in the range of 0.5 to 2.0 moles but the most preferable ratio is1:1[57,58]. Thin flim is formed after evaporation of the solvent. Futher hydration of the flim leads to formation of phytosomal suspension. The formed phytosomes will be collected by precipitation technique. The collected phytosomes are futher subjected to drying by lyophilisation method [59,60]. The entire preparations are illustrated in schematic way for better understanding to the users (Fig 1.6).

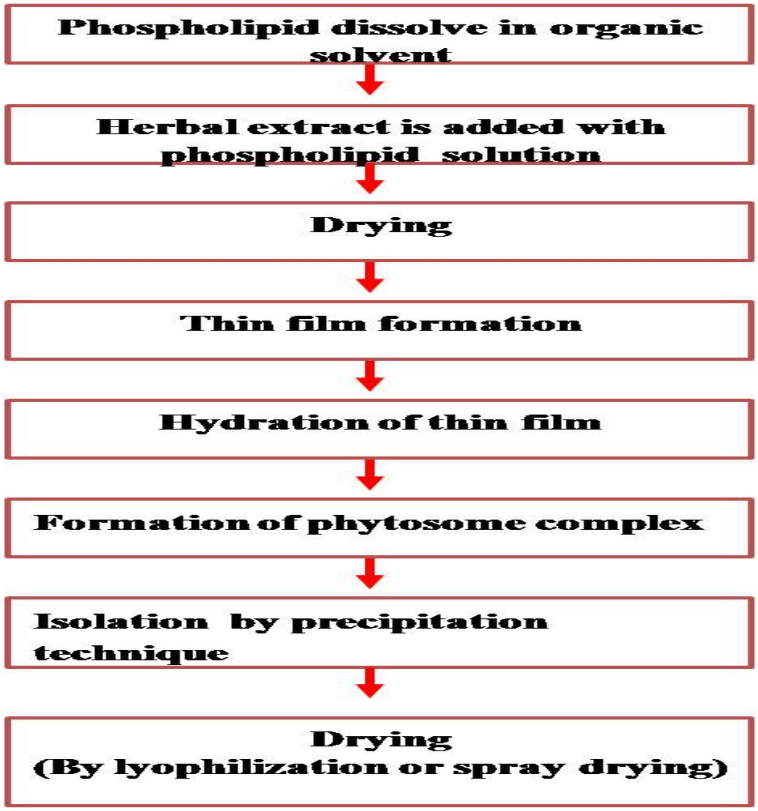


Figure 1.6: Schematic illustration of preparation of nanophytosomes.

1.4.3. Characterization and Evaluation of Phytosomes

The characterization techniques for the evaluation of phytosomes are as follows

i. **Vesicle size and Zeta potential:** The particle size and zeta potential can be determined by DLS using a computerized inspection system.
ii. **Surface morphology analysis:** By using scanning electron microscopy (SEM) the surface morphology analysis of phytosomes can be determined.
iii. **Transition temperature:** By using differential scanning calorimetry the transition temperature of the vesicular lipid system can be determined.
iv. **Surface tension measurement**: Du novy ring densitometer is used to find out the surface tension activity of a drug dissolved in aqueous solution.
v. **Entrapment efficiency**: By using ultracentrifugation technique the drug entrapment ability of phytosomes can be measured.
vi. **Drug content**: Drug content present in the phytosomes can be determined by High performance liquid chromatographic method or any other spectroscopic methods.

vii. Stability studies: Stability studies were carried out for two months on the optimized formulation of phytosomes. For stability study the optimized formulation were placed in humidity chamber (sonar) at 75%RH,45^{0}c. After two months, the formulation was evaluated for weight variation, hardness, friability, disintegration and percentage drug content[61-64].

1.4.4. Spectroscopic Evaluations

The spectroscopic evaluation provides more information about phytosomes. They are as follows:

i)FT-IR: The FT-IR spectra data will be taken to determine the structure and chemical shift of the extract, phosphotidylcholine and phytosomes.

ii)^{1}H-NMR: The ^{1}H-NMRspectra is used to determine the development of complex formed between active phytoconstituents and phosphotidylcholine molecules. In non polar solvents, there will be an evident change in ^{1}H-NMR signal commencing from atoms included in the complex formation. The signals from protons are broadened. In phospholipids there is broadening of signals whereas the singlet correlative to the N- trimethyl portion of choline yields an upfield shift.

iii)^{13}C-NMR: The ^{13}C-NMR of phytosomes, when recorded at room temperature all the carbons in phytoconstituents are unobservable. The signals equivalent to the choline and glycerol portion was broadened, whereas some are shifted and most of the resonance of the fatty acids chains maintains their initial sharp lines[65,66].

1.4.5. Commercial Products in Market

To date, very few products has came in to market and said to be commercially available. The list of available **(Table 1.4)** components is enlisted here for readers.

Table 1.4:Marketed preparation of phytosomes

S.No.	Nanophytosomes	Phytoconstituents	Therapeutic Applications
1.	Silybin Phytosome	Silybin from Silybum marianum	Hepatoprotective, antioxidant for liver and skin
2.	Ginkgo Phytosome	24% ginkgo flavonoids	Protects brain and vascular linings, anti-skin Ageing
3.	Ginseng Phytosome	37.5% ginsenosides	Nutraceuticals, immunomodulator
4.	Green Tea Phytosome	Epigallocatechin	Nutraceutical, systemic antioxidant, anticancer
5.	Grape Seed Phytosome	Procyanidins	Nutraceutical, systemic antioxidant, cardioprotective
6.	Hawthorn Phytosome	Flavonoids	Nutraceutical, cardio-protective and antihypertensive.
7.	Olive oil Phytosome	Polyphenols	Antioxidant, anti-inflammatory, antihyperlipidemic.
8.	Echinacea Phytosome	Echinacosides	Nutraceutical, immunomodulatory
9.	Centella Phytosome	Terpenes	Vein and Skin disorders
10.	Palmetto berries Phytosomes	Fatty acids, alcohols and sterols	Non-cancerous prostate enlargement

PLANT PROFILE

2. PLANT PROFILE

Figure 2.1: *Momorica Charantia*

2.1. Taxonomy of *Momorica Charantia*

The botanical classification of *Momorica Charantia* is as following,

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Cucurbitales
Family : Cucurbitaceae
Genus : Momordica
Species : Charantia

2.2. Vernacular Names of *Momordica Charantia*

English : Bitter gourd
Tamil : pakal/paavakka
Chinese : kugua
Japanese : nigauri
Okinawan : goya
Gujarati : karela
Marathi : karala
Hindustani : karavila
Malayalam : kaipakka/paavakka
Kannada : hagala
Telugu : kakarakaya
Assamese : kerela

2.3. Morphology

Leaves: simple, usually palmately 5-7 lobed, tendril sun branched or 2 branched. The herbaceous,tendril bearing vine grows to 5 m. It bears simple, alternate leaves 4–12 cm across, with 3–7 deeply separated lobes.

Fruit: ovoid, ellipsoid, or spindle shaped, usually ridged or warty, dehiscent irregularly as a 3 valves fleshy capsule or indehiscent. The fruit has a distinct warty looking exterior and an oblong shape. It is hollow in cross-section, with a relatively thin layer of flesh surrounding a central seed cavity filled with large flat seeds and pith. Seeds and pith appear white in unripe fruits, ripening to red; the flesh is crunchy and watery in texture, similar to cucumber, chayote or green bell pepper.

Flowers: Staminate flowers usually solitary on a bracteate scape, hypanthium shallow, calyx 5 lobed, petals 5,usually yellow, distinct, 1-3 with incurved scales at base, stamensusually 3, inserted toward base of hypanthium, filaments distinct, broad, anthers distinct or coherent, 2 ofthem dithecal, the other monothecal, cells curved or flexuous; pistillate flowers usually solitary on a bracteatescape, hypanthium ovoid to spindle shaped, perianthusually smaller than in staminate flowers, staminodesabsent or 3, ovules numerous, horizontal, stigmas 3, 2 lobed. Seeds few to numerous, ovate, usually sculptured[67].

2.4. Plant Parts Used

The *Momodrica Charantia* leaves are used.

2.5. Distribution

The original home of the species is not known, other than that it is a native of the tropics. Bitter melon grows in tropical areas, including parts of the Amazon, east Africa, Asia, and the Caribbean. It is widely grown in India and other parts of the Indian subcontinent, Southeast Asia, China, Africa, and the Caribbean.

2.6. Phytochemical Constituents

Momordica charantia has a non-nitrogenous neutral principle charantin, and on hydrolysis gives glucose and asterol. The fruit pulp of *momordica charantia* has soluble pectin but no free pectic acid. Galactouronic acid is also obtained from the

pulp. *Momordica charantia* fruits glycosides, saponins, alkaloids, reducing sugars, resins, phenolic constituents, fixed oil and free acids. The presence of anun identified alkaloid and 5-hydroxytryotamine is also reported. The 5HT content is reported to be present. Theether extract residue of the alcoholic concentrate from the leaves of *Momordica charantia* is reported to reveal hypoglycemic activity comparable to that of tolbutamide. The pure protein termed as P-insulin extracted from momordica charantia fruits in crystalline form is also tested[68].

2.7. Traditional Uses

In traditional medicine of India, different parts of the plant are used as claimed treatments for diabetes (particularly Polypeptide-p, an insulin analogue), and as a stomachic, laxative, antibilious, emetic, anthelmintic agent, for the treatment of cough, respiratory diseases, skin diseases, wounds, ulcer, gout, and rheumatism. *Momordica charantia* has a number of purported uses including cancer prevention, treatment of diabetes, fever, HIV and AIDS, and infections. For cancer prevention, HIV and AIDS, and treatment of infections, there is preliminary laboratory research, but no clinical studies in humans showing a benefit. In 2017, the University of Peradeniya researchers revealed that bitter gourd seeds can be potentially used to destroy cancer cells and they were successfully administered to patients inK andy General Hospital Cancer Unit. The Memorial Sloan Kettering Cancer Center concludes that bitter melon "cannot be recommended as a replacement therapy for insulin or hypoglycaemic drugs"[69].

REVIEW OF LITERATURE

3. REVIEW OF LITERATURE

1. Mamdouh Mali *et al*., (2017) reported in spite of the wide facilities for controlling cancer growth, there are little drugs to inhibit its metastasis or prevent its angiogenesis. Discovering such natural or synthetic multi-targeted agent that might strike different targets is considered as a vital goal for tumor controlling. In a previous study, the chemo protective effect of methanol extract of *Momordica charantia* (MEMC) on albino western rats bearing hepato carcinogenesis was evaluated. The mechanism by which MEMC exert its anticancer properties was unknown. Therefore, we aimed in this study to investigate the possible role of MEMC as anti-proliferative, anti-angiogenic and anti-metastatic agent to exert its chemo protective effect. MEMC treatment significantly decreased Cox-2, VEGF, HDAC and MMP-2,-9 and increased Casp-3,−8 as compared to DENA group, which demonstrated that the anticancer effect of MEMC may be through the inhibition of angiogenesis, proliferation and metastasis and the activation of apoptosis[70].

2. Sur *et al*., (2017) reported head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer worldwide, and tobacco is one of the most common factors for HNSCC of the oral cavity. We have previously observed that bitter melon (*Momordica charantia*) extract (BME) exerts anti proliferative activity against several cancers including HNSCC. In this study, we investigated the preventive role of BME in 4-nitroquinoline 1-oxide (4-NQO) carcinogen-induced HNSCC. We observed that BME feeding significantly reduced the incidence of 4-NQO-induced oral cancer in a mouse model. Histologic analysis suggested control 4-NQO-treated mouse tongues showed neoplastic changes ranging from moderate dysplasia to invasive squamous cell carcinoma, whereas no significant dysplasia was observed in the BME-fed mouse tongues. We also examined the global transcriptome changes in normal versus carcinogen-induced tongue cancer tissues, and following BME feeding[71].

3. Bhattacharya *et al*., (2017) reported natural killer (NK) cells are one of the major components of innate immunity, with the ability to mediate antitumor activity. Understanding the role of NK-cell-mediated tumor killing in controlling of solid tumor growth is still in the developmental stage. We have shown recently that bitter melon extract (BME) modulates the regulatory T cell (Treg) population in head and neck

squamous cell carcinoma (HNSCC). However, the role of BME in NK-cell modulation against HNSCC remains unknown. In this study, we investigated whether BME can enhance the NK-cell killing activity against HNSCC cells. Our results indicated that treatment of human NK-cell line (NK3.3) with BME enhances ability to kill HNSCC cells[72].

4. Vermont Dia *et al* ., (2016) reported *momordica charantia* is a perennial plant with reported health benefits. BG-4, a novel peptide from *Momordica charantia,* was isolated, purified and characterized. The trypsin inhibitory activity of BG-4 is 8.6 times higher than purified soybean trypsin inhibitor. The high trypsin inhibitory activity of BG-4may be responsible for its capability to cause cytotoxicity to HCT-116 and HT-29 human colon cancer cells with ED50 values of 134.4 and 217.0 µg/mL after 48 h of treatment, respectively. The molecular mechanistic explanation in the apoptosis inducing property of BG-4 is due to reduced expression of Bcl-2 and increased expression of Bax leading to increased expression of caspase-3 and affecting the expression of cell cycle proteins p21 and CDK2. This is the first report on the anti-cancer potential of a novel bioactive peptide isolated from *Momordica charantia in vitro* supporting the potential therapeutic property of BG-4 against colon cancer that must be addressed using *in vivo* models of colon carcinogenesis[73].

5. Mohammed Alshehri *et al*., (2016) reported natural products are the best source for various medicinal drugs. Regardless investigate the toxic effect of these plant extracts, the results will still unsafe and unacceptable. This study aimed to identify anti-cancer activity and cytotoxicity effect of *Momordica charantia* extract on different cancer cell line. To archive the aim of this study 3 different cancer cell lines (HCT116, MCF-7, and HepG2) were treated with different *Momordica charantia* extract doses (from 0-100µg) for each cell line. IC50, cell viability, apoptosis, were evaluated. The effect of *Momordica charantia* extract was highly significant in HepG2 cells than HCT116cell as well as MCF-7 which showing the IC50 of *Momordica charantia* extract in HepG2 was 0.77 µg/ml whilein HCT116 was 0.81µg/ml and was 1.35µg/ml in MCF-7 cells respectively. Also, the effect of the *Momordica charantia* extract was more potent in HCT116 compared to MCF-7 cells[74].

6. Mingo Yung *et al*., (2015) reported Acquired chemoresistance is a major obstacle in the clinical management of ovarian cancer. Therefore, searching for alternative therapeutic modalities is urgently needed. Bitter melon (*Momordica charantia*) is a traditional dietary fruit, but its extract also shows potential medicinal values in human diabetes and cancers. Here, we sought to investigate the extract of bitter melon (BME) in antitumorigenic and cisplatin-induced cytotoxicity in ovarian cancer cells. Three varieties of bitter melon were used to prepare the BME. Ovarian cancer cell lines, human immortalized epithelial ovarian cells (HOSEs), and nude mice were used to evaluate the cell cytotoxicity, cisplatin resistance, and tumor inhibitory effect of BME. The molecular mechanism of BME was examined by Western blotting. Cotreatment with BME and cisplatin markedly attenuated tumor growth *in vitro* and *in vivo* in a mouse xenograft model, whereas there was no observable toxicity in HOSEs or in nude mice *in vivo*[75].

7. Shobha *et al*., (2015) reported to estimate the total phenol content (TPC)of the ethanolic extract of *Momordica charantia* (EEMC)whole fruit and to study the cytotoxic activity of this extract against cell lines representing carcinomas of cervix and breast. Cervical and breast carcinoma cell lines (HeLa and MCF-7) were procured from National Center for Cell Sciences, Pune, and cultured in Dulbecco's modified eagle medium (DMEM)supplemented with 10% fetal bovine serum (FBS) and1 mM L-glutamine. EEMC was prepared by graded ethanol fractionation method and the TPC determined using Folin–Ciocalteu assay. For cytotoxicity studies,5000 cells/well in 100 µl DMEM-10% FBS medium were seeded in a 96 well plate; and treated with increasing concentration of EEMC. Efficacy of EEMC was determined by measuring the cell number using sulforhodamine B assay[76].

8. Gunasekar Manoharan *et al*., (2014) reported prior to the availability of chemotherapeutic agents, dietary measures, including traditional medicines derived from plants, were the major forms of cancer treatment. One such plant is Momordica charantia (Family: Cucurbitaceae), whose fruit is known as corilla or bitter gourd/melon. M. charantia possesses anti-carcinogenic properties and it can modulate its effect via xenobiotic metabolism and oxidative stress. This study investigated the anti-cancer effect of an active water-soluble extract (s) of M. charantia on cell viability and its cellular mechanism(s) of action in inducing cell

death. Both time course (800 µg/ml) and dose- dependent (200 µg/ml800 µg/ml) experiments were performed treating six different cancer cell lines, namely 1321N1, Gos-3, U87-MG, Sk Mel, Corl -23, Weri Rb-1 and normal L6 muscle cell line with the crude fruit extract for 24-48 hours at 37 °C. Cell viability was measured using the MTT assay[77].

9. Sara Abozaid *et al*., (2014) reported Cancer today represents a significant public health problem worldwide and the challenge is to produce cost effective drugs. Recently, a surge of progress was achieved in the area of cytotoxic proteins. Eleven crude protein extracts, from different plants, were tested for anticancer activities against four cell lines; HepG2, Caco-2, HEP-2,and HeLa cells using MTT assay. Out of the tested extracts, *Momordica charantia* showed the highest anticancer potency of all tested protein fractions that is superior than that of 5-FU. Furthermore, its anticancer activity was characterised by the selective growth inhibitory effect on epithelial derived cell lines, HeLa, Caco-2 andHEP-2, but not in HepG2. On the other hand, *Lactuca sativa* protein fraction exhibited a potent and a selective anti cancer activity against HepG2 that could be used as a lead for further investigations in the area of liver cancer therapy. In conclusion, although these findings are very promising and may open an avenue towards an economic plant-based selective anticancer drug, the exact chemical nature of active fractions remains to be fully explored and elucidated[78].

10. Chia-Jung Li, *et al*., (2012) reported Plants are an invaluable source of potential new anti-cancer drugs. Momordica charantia is one of these plants with both edible and medical value and reported to exhibit anticancer activity. To explore the potential effectiveness of Momordica charantia, methanol extract of Momordica charantia (MCME) was used to evaluate the cytotoxic activity on four human cancer cell lines, Hone-1 nasopharyngeal carcinoma cells, AGS gastric adenocarcinoma cells, HCT-116 colorectal carcinoma cells, and CL1-0 lung adenocarcinoma cells, in this study. MCME showed cytotoxic activity towards all cancer cells tested, with the approximate IC_{50} ranging from 0.25 to 0.35 mg/mL at 24 h. MCME induced cell death was found to be time-dependent in these cells. Apoptosis was demonstrated by DAPI staining and DNA fragmentation analysis using agarose gel electrophoresis[79].

11. Pitchakarn *et al*., (2012) reported in this study, we focused on the invitro effects of kuguacin J, purified component of bitter melon(momordica charantia) leaf extract(BMLE) on androgen- independent human prostate cancer cell line PC3 and the invivo effect of dietary BMLE on prostate carcinogenesis using a PC3-xenograph model. Interestingly, KuJ also dramatically decreased the level of survivin expressed by PC3 cells. In addition , KuJ exerted anti-invasive effects on PC3 cells. In addition ,KuJ inhibited secretion of the active forms of MMP-2,MMP-9 and uPAby PC3 cells. In addition KuJ treatment significantly decreased the expression of membrane type 1-MMP (MT1-MMP) by PC3cells. In vivo , 1% and 5% BMLE in the diet resulted in 63% and 57% inhibition of PC3 xenograph growth without adverse effect on host body weight. Our results suggest that KuJ is a promising new candidate chemo preventive and chemotherapeutic agent for prostate cancer[80].

12. Peng Ru *et al*., (2011) reported prostate cancer remains the second leading cause of cancer deaths among American men. Earlier diagnosis increases survival rate in patients. However, treatments for advanced disease are limited to hormone ablation techniques and palliative care. Thus, new methods of treatment and prevention are necessary for inhibiting disease progression to a hormone refractory state. One of the approaches to control prostate cancer is prevention through diet, which inhibits one or more neoplastic events and reduces the cancer risk. In this study, we have initially used human prostate cancer cells, PC3 and LNCaP, as an in vitro model to assess the efficacy of bitter melon extract (BME) as an anticancer agent. We observed that prostate cancer cells treated with BME accumulate during the S phase of the cell cycle and modulate cyclin D1, cyclin E, and p21 expression. Treatment of prostate cancer cells with BME enhanced Bax expression and induced PARP cleavage[81].

13. Pitchakarn *et al*., (2011) reported in this study, we focused on the effects of a bitter melon(momordica charantia) leaf extract(BMLE) and a purified component, kuguacin J, on androgen dependent LNCaP human prostate cancer cells. Both treatments exerted growth inhibition through G1 arrest and induction of apoptosis. Down –regulation of p53 by RNA interference indicated that BMLE and KuJ inhibited cell growth partly through p53-dependent cell cycle arrest and apoptotic pathways. Both BMLE and KuJ caused less toxicity in a normal prostate cell line, PNT1A. Our

results suggest that BMLE and a purified component, KuJ, from its diethyl ether fraction could be promising candidate new anti neoplastic and chromo preventive agents for androgen-dependent prostate cancer and carcinogenesis[82].

14. **Wei-Hsiung Yang *et al*., (2011)** reported adreno cortical carcinomas are rare but present with extremely poor prognosis. One of the approaches to control cancer progression and reduce cancer risk is prevention through diet. In this study, we have used human and mouse adreno cortical cancer cells as an *in vitro* model to assess the efficacy of bitter melon extract (BME) as an anticancer agent. The protein concentrations of BME and other extracts were measured before use. First, BME treatment of adreno cortical cancer cells resulted in a significantly dose-dependent decrease in cell proliferation. However, we did not observe an anti proliferative effect in adreno cortical cancer cells treated with extracts from blueberry, zucchini, and acorn squash. Second, apoptosis of adreno cortical cancer cells was accompanied by increased caspase-3 activation and poly(ADP-ribose) polymerase cleavage. Third, BME treatment decreased the key proteins involved in steroidogenesis in adrenocortical cancer cells[83].

15. Ray *et al*., (2010) reported Breast cancer is one of the most common cancers among women in the United States. Although there are effective drugs for treating advanced stages of breast cancers, women eventually develop resistance. One of the approaches to control breast cancer is prevention through diet, which inhibits one or more neoplastic events and reduces cancer risk. In this study, we have used human breast cancer cells, MCF-7 and MDA-MB-231, and primary human mammary epithelial cells as an in vitro model to assess the efficacy of bitter melon (Momordica charantia) extract (BME) as an anticancer agent. BME treatment of breast cancer cells resulted in a significant decrease in cell proliferation and induced apoptotic cell death. Apoptosis of breast cancer cells was accompanied by increased poly(ADP-ribose) polymerase cleavage and caspase activation. Together, these results show that BME modulates signal transduction pathways for inhibition of breast cancer cell growth and can be used as a dietary supplement for prevention of breast cancer[84].

16. Pornsiri Pitchakarn *et al*., (2010) reported cancer metastasis is a major cause of death in cancer patients, with invasion as a first step greatly contributing to the failure of clinical treatments. Any compounds with an inhibitory influence on this process are therefore of prime interest. Momordica charantia is widely consumed as a vegetable and especially as a folk medicine in Asia. Here , we investigated the anti –invasive effects of bitter melon leaf extract (BMLE) on a rat prostate cancer cell line(PLS10) in vitro and in vivo. The results indicated that non-toxic concentrations of BMLE significantly inhibited the migration and invasion of cells in vitro. The results of zymography showed that BMLE inhibited the secretion of MMP-2, MMP-9 and urokinase plasminogen activator from PLS10[85].

17. Li M *et al*., (2009) reported ribosome-inactivating proteins (RIPs) are a family of enzymes that depurinate rRNA and inhibit protein biosynthesis. Here we report the purification, apoptosis-inducing activity, and polyethylene glycol (PEG) modification of RIP from the bitter melon seeds. The protein has a homogenous N-terminal sequence of NAsp- Val-Ser-Phe-Arg. Moreover, the RIP displayed strong apoptosis-inducing activity and suppressed cancer cell growth. This might be attributed to the activation of caspases-3. To make it available for *in vivo* application, the immunogenicity of RIP was reduced by chemical modification with 20 kDa (mPEG)(2)-Lys-NHS. The inhibition activity of both PEGylated and non-PEGylated RIP against cancer cells was much stronger than against normal cells, and the antigenicity of PEGylated RIP was reduced significantly[86].

18. Kobori *et al*., (2008) reported bitter gourd (Momordica charantia L.) pericarp, placenta, and seed extracts were previously shown to induce apoptosis in HL60 human leukemia cells. To determine the active component that induces apoptosis in cancer cells, bitter gourd ethanol extract was fractionated by liquid-liquid partition and silica gel column chromatography. Several fractions obtained by silica gel column chromatography inhibited growth and induced apoptosis in HL60 cells. (9Z,11E,13E)-9,11,13-Octadecatrienoic acid (alpha-eleostearic acid) is known to be the major conjugated linolenic acid in bitter gourd seeds. Therefore, the effect of alpha-eleostearic acid on the growth of some cancer and normal cell lines was examined. alpha-Eleostearic acid strongly inhibited the growth of some cancer and fibroblast cell lines, including those of HL60 leukemia and HT29 colon carcinoma[87].

AIM AND OBJECTIVES

4. AIM AND OBJECTIVE

4.1. Aim

The aim of our study is to prepare nanophytosomes of aqueous extract of leaves of *Momordica Charantia* and to evaluate them for its *in vitro* anti cancer activity in a MDA-MBhuman breast cancer cell lines.

4.2. Objectives

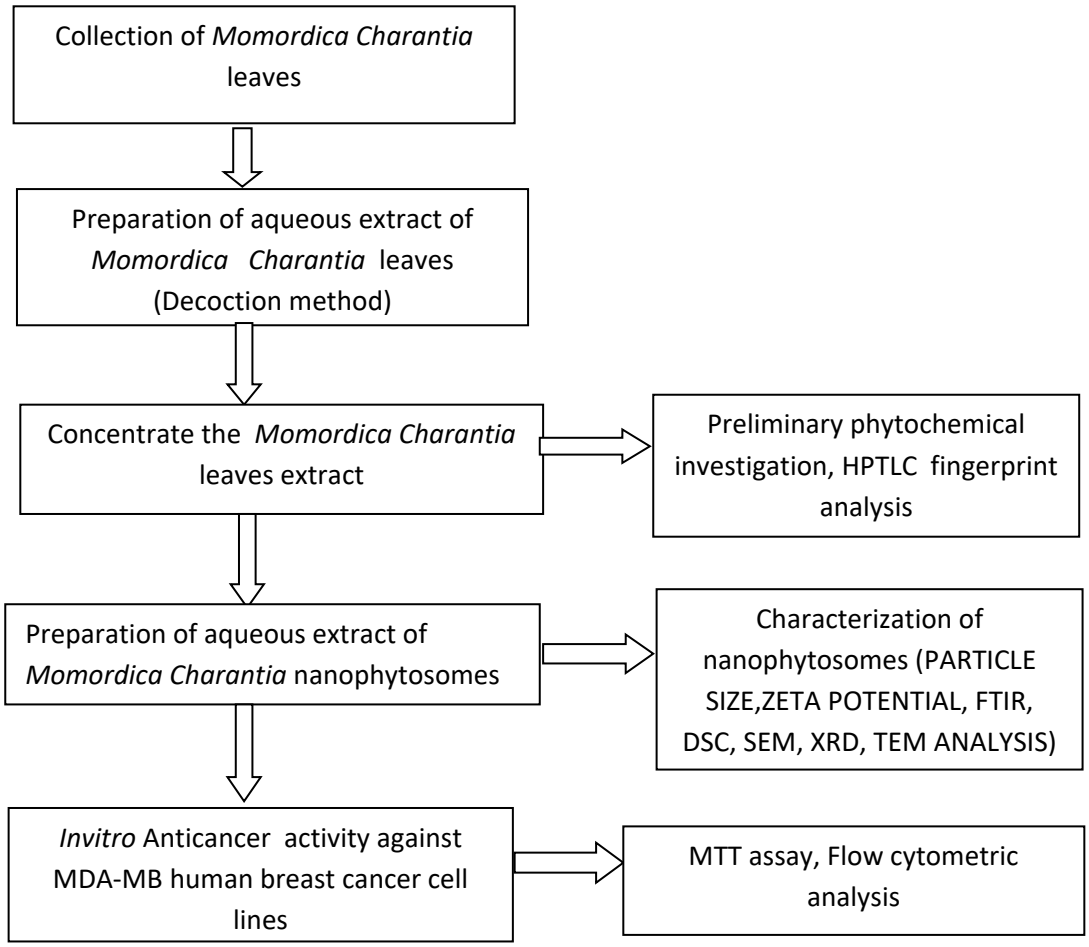
The objectives of the present research work focused on the investigation of phyto chemical constituents of aqueous extract, preparation of nanophytosomes of aqueous extract and study of anti cancer activity in a MDA-MBhuman breast cancer cell lines.

The specific objectives are as follows,

1. Collection of *Momordica Charantia* leaves.
2. Processing of leaves to prepare aqueous extract using decoction method.
3. Preliminary Phytochemical Investigation of aqueous extract of leaves of *momordica charantia* .
4. Preparation of nanophytosomes of aqueous extract of *momordica charantia.*
5. Characterization of Aqueous extract of *Momordica Charantia* nanophytosomes.
6. Testing of *in vitro* anti cancer activity of extract and nanophytosome of leaves of *momordica charantia* against MDA-MBhuman breast cancer cell lines.

4.3. Plan of Work

It is planned to carry out this work as outline below.



MATERIALS AND METHODS

5. MATERIALS AND METHODS

5.1.Materials

Cholesterol were obtained from Loba Chem lab pvt ltd, Maharashtra. Phosphotidyl Choline is obtained from Lipoid pharma pvt ltd , Germany. Chloroform was obtained from Sisco Research lab, pvt ltd, Mumbai. All reagents and glass ware used are of analytical grade.

5.2.Methodology

5.2.1. Collection and Processing of *Momordica Charantia* Plant Material

Momorica Charantia plants were collected locally from the village of Muhavur, Srivilliputhur (Virudhunagar Dist,Tamilnadu). The leaves were separated from the plant and the leaves were washed with water and then again washed with chloroform to remove soil particles and the leaves were spread and dried in the shade for 4 days.

5.2.2 Preparation of Aqueous Extract of *Momordica Charantia*

The *momordica charantia* leaves were subjected to size reduction by trituration by using mortar and pestle to make into fine powder. Weigh 10g of powder and it is dissolved in a 250ml of boiling water. Then the mixture of powder and water is placed in water bath at 40^0c for 1 hour. Then it is filtered by using whatmann filter paper. Then the filtrate was concentrated on water bath at 40^0c for 2days.Then finally extract was collected and stored in desicator at room temperature.

5.2.3. Preliminary Phytochemical Analysis [89]

The aqueous extract of *momorica charantia* obtained was subjected to qualitative analysis to test the presence of various phytochemicals like alkaloids, flavonoids, steroids, phenols, proteins and amino acids, terpenoids,anthraquinones and quinones etc.

Procedure

I. Test for Alkalaoids

A. Mayer's test

A fraction of extract was treated with mayers test reagent(1.36 g of mercuric chloride and 5g of potassium iodide in 100ml water) and observed for the formation of cream coloured precipitate.

B. Wagner's test

A fraction of extract was treated with wagner's test reagent (1.27 g f iodide and 2g of potassium iodide in 100 water) and observed for the formation of reddish brown coloured precipitate.

C. Hager's test

A few ml of extract was treated with hager's test reagent(saturated aqueous solution of picric acid) and observed for the formation of prominent yellow coloured precipitate.

II. Test for Flavanoids

A. NaoH test

A small amount of extract was treated with aqueous NaoH and HCl, observed for the formation of yellow orange colour.

B. H_2SO_4 test

A fraction of extract was treated with concentrated H_2SO_4 and observed for the formation of orange colour.

III. Test For Sterols

A. Liebermann-Burchard test

A fraction of extract was treated with chloroform, acetic anhydride and drops of concentrated H_2SO_4 was added and observed for the formation of pink or red colour.

IV.Test for Phytosterols

A. Salkowski test

The fraction of extract was dissolved in few drops of acetic acid and three drops of acetic anhydride was added followed by few drops of concentrated sulfuric acid. Bluish green colour was formed shows the presence of phytosterol.

V. Test for Terpenoids

A. Liebermann-Burchard test

A fraction of extract was treated with chloroform, acetic unhydride and drops of concentrated H_2SO_4 was added and observed for the formation of greyish colour.

VI. Test for Proteins and Amino Acids

A. Ninhydrin test (aqueous)

A fraction of extract was treated with aqueous ninhydrin and observed for the formation of blue colour, indicating presence of amino acids or purple colour indicating the presence of protein.

B. Ninhydrin test (acetone)

Ninhydrin was dissolved in acetone,the extract was treated with ninhydrin and observed for the formation of purple colour.

C. Biuret test

The extract was heated in distiiled water and filtered. The filtrate is treated with 2%copper sulphate solution,to this added 95%ethanol and potassium hydroxide and observed the formation of pink colour.

VII. Test for Anthraquinones

A. Borntrager's test

About 50mg of powdered extract was heated with 10%ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken

with diethyl ether. The ether extract was further extracted with strong ammonia; pink or deep red colour of aqueous layer indicate the presence of anthraquinones.

VIII. Test for Phenols

A. Ferric chloride test

A fraction of extract was treated with 5% ferric chloride and observed for the formation of deep black or blue colour.

B. Liebermann's test

The extract was heated with sodium nitrite, added H_2SO_4 solution diluted with water and excess of dilute NaoH was added and observed for the formation of deep red or green or blue colour.

IX. Test for Tannins

A. Ferric chloride test

A small amount of extract was treated with ferric chloride solution and observed for the formation of bluish black colour.

X. Test for saponins

A. Foam test

A small amount of extract was added with water and shaken well and observed for the formation of foams.

5.2.4. HPTLC Fingerprint Analysis[90]

HPTLC finger printing analysis of aqueous extract of *momordica charantia* leaves

Preliminary phytochemical analysis for *momordica charatia leaves* extracts were carried out as per the protocol mentioned in Harbore,1998 (Paterson, 1999). For HPTLC (silica gel G 60F254 TLC plates of E. Merck, layer thickness 0.2 mm) fingerprint analysis was established for aqueous extracts of *momordica charantia leaves*. HPTLC was performed on (10 cm X 10 cm) aluminum backed plates coated

with silica gel 60F254 (Merck, Mumbai, India). Standard solution of quercetin and test were applied to the plates as bands 8.0 mm wide, 30.0 mm apart, and 10.0 mm from the bottom edge of the same chromatographic plate by use of a Camag (Muttenz, Switzerland) Linomat V sample applicator equipped with a 100 µL Hamilton (USA) syringe. Ascending development to a distance of 80 mm was performed at room temperature (28±2^{o}c), with toluene: ethyl acetate: formic acid [(5: 4: 1) (v/v/v)], as mobile phase, in a Camag glass twintrough chamber previously saturated with mobile phase vapour for 20 min. Quercetin of (100 µg/mL) was used as standard (Vijayalakshmi, Ravichandiran, Malarkodi, Nirmala, & Jayakumari, 2012).

5.2.5. Preparation of Nanophytosomes: Thin film hydration method

Accurately weighed quantity of egg lecithin and cholesterol were dissolved in 10 ml of chloroform in round bottom flask (RBF) and sonicated for 10 min using bath sonicator. Organic solvent removal is done by Rotary evaporator (45-50°C). After complete removal of solvent thin layer of phospholipids mixture was formed. This film was hydrated with Aqueous extract of *momorica charantia* leaves in rotary evaporator (37-40°C for 1 hour). After hydration, mixture of lipid and plant extract was sonicated for 40 minutes in presence of ice bath for heat dissipation. Then prepared phytosomes were filled in amber colored bottle and stored in freezer (2-8 ^{0}C) until used. The different phytosome complexes of momorica charantia F1, F2, F3 & F4 containing molar ratio of 1:0.5:1, 2:1:1, 1:0.5:2 and 2:1:2 of Egg lecithin, Cholesterol and *momorica charantia* were prepared as mentioned in (Table 5)

Composition of phytosome formulation of aqueous extract of *momorica charantia* leaves.

Table 1.5: Composition of phytosome formulation of aqueous extract of *momorica charantia* leaves

Phytosomes	Molar ratio (EL:CL:EX)	chloroform
F1	1:0.5:1	10ml
F2	2:1:1	10ml
F3	1:0.5:2	10ml
F4	2:1:2	10ml

EL: Egg Lecithin; CL: Cholesterol; EX: Extract

5.2.6. Characterization of Nanophytosomes[91]

5.2.6.1. Particle size

Particle size of prepared nanophytosomes was analyzed by photon correlation spectroscopy using a Shimadzu particle size analyzer (SALD 2101, Japan). Diluted nanophytosomal suspension was placed into the sample dispersion unit while stirring at room temperature (in order to reduce the inter particle aggregation). All analyses has been performed in triplicate.

5.2.6.2. Zeta potential determination

Surface charge of *momorica charantia* -loaded nanophytosomes was determined using a Malvern Zetasizer (Nano-ZS, UK). Samples were diluted (50 folds) using distilled water and then analysis was performed at 25 ºC and 149 watt. The average three zeta potential determination of the nanophytosomes was calculated.

5.2.6.3. Fourier Transformer-Infra Red Spectroscopy (FTIR) analysis

FT-IR spectral data can be taken to determine the structure and chemical stability of pure drug in the presence of excipients, physical mixture of egg lecithin and cholesterol, physical mixtures and nanophytosomal formulation. were evaluated by FT-IR analysis. The spectroscopic evaluation of the formed complex can be

confirmed by FTIR simply by mparing the spectrum of the complex and the individual components and that of the mechanical mixtures. Samples were mixed with dry crystalline KBr in a ratio of 1:100 and pellets were prepared. The mixture was grounded or triturated into fine powder using an agate mortar before compressing into KBr disc. Each KBr disc was scanned at 4 mm/s at a resolution of 2. FTIR can also be considered as a valuable tool in confirming the stability of the phytosomal complex. FT-IR spectra were obtained using a FT-IR spectrometer . Spectral scanning can be done in the range between 4000-400 $^{cm-1}$.

5.2.6.4. Differential Scanning Calorimetry (DSC) analysis

Thermodynamical techniques are applied for determining the thermal stress of medicinal compounds of the excipients as well as their interactions during the formulation process. The thermal analysis of the *momordica charantia*, physical mixture of egg lecithin and cholesterol, physical mixture of egg lecithin and cholesterol and extract of momorica charantia were placed in the aluminum crimp cell and heated at 10^0C/min from 0 to 400^0C in the atmosphere of nitrogen (TA Instruments, USA, model DSC Q10 V24.4 Build 116). Peak transition onset temperatures were recorded by means of an analyzer. Momorica charantia leaves extract, phospholipon and phytosome were placed in the aluminum crimp cell and heated at 100C/min from 0 to 400 0C in the atmosphere of nitrogen (TA Instruments, USA, Model DSC Q10 V24.4 Build 116). Peak transition onset temperatures were recorded by means of an analyzer.

5.2.6.5. Scanning Electron Microscopy (SEM) analysis

Scanning electron microscopy has been used to determine particle size distribution and surface morphology of the complexes. Samples were studied using JEOL JSM-6360 Scanning microscope (Japan). Approximately 5 µL of the nanophytosomal suspension was transformed to a cover slip, which in turn was mounted on a specimen tab. The samples were allowed to dry at room temperature. Then the particle size of the formulation was viewed and photographed using Scanning Electron Microscope (Sigma, Carl Zeiss). The particles were coated with platinum by using vaccum evaporator and thus, the coated samples were viewed and photographed in JEOL JSM-6701F Field Emission SEM. Digital images of

phytosome complex of momorica charantia were taken by random scanning of the stub at different magnifications.

5.2.6.6. X-Ray Diffraction (XRD) analysis

XRD is a unique method in determination of crystallinity of a compound and when properly interpreted, by comparison with drug XRD pattern before formulation., allows the identification of the drug crystalline changes. XRD was done on pure extract, physical mixtures of egg lecithin and cholesterol, physical mixtures and nanophytosome to see the crystallinity in the substance. Sample was scanned in the angular range of 50 - 800 in a PHILIPS XPert Pro X-Ray Diffractometer. Dried powder sample was kept in sample holder (20 mm × 15mm × 2mm) which was fitted into the instrument and X-ray was passed through the sample.

5.2.6.7. Transmission Electron Microscopy (TEM) analysis

Vesicles morphology of nanophytosome was observed visually with a JEOL JEM 1400 (Japan) Transmission Electron Microscopy (TEM). A total volume of 10 ml sample was dispersed before the sample was analyzed. The mixture was then stirred and a drop of the sample was placed on the specimen. The 400 mesh grid was placed over the specimens and allowed to stand for 1 minute. Residual droplets on the grid were cleaned using a filter paper. A drop of 5 uranyl acetate was dropped over the grid and the rest of the excess solution was removed using a filter paper. The grid was left for 30 minutes and the films were then viewed on a transmission electron microscope and photographed.

5.3. *IN-VITRO* STUDIES

5.3.1. Cell Culture

The MDA-MBcells were grown and maintained in a humified incubator at 37˚C under 5% Co_2 atmosphere in MEM medium (Minimal Essential Media) supplemented with TPVG & 10% Fetal calf serum and (100 units/ml penicillin).For experimental purpose cells were plated in 48 well plates (at a density of 1x104cells/ ml). After 25hrs incubation period , to allow cell attachment, the cells were treated with fresh medium containing different concentration of aqueous extract and nanophytosomes of

leaves of *Momordica Charantia* ranging from 10-100 µg/ml, dissolved in DMSO and incubated for 48hrs and it is used for study of MTT assay[92].

5.3.2. MTT Assay

It is a sensitive, quantitative and reliable colorimetric assay that measure viability, proliferation and activation of cells. The assay is based on the capacity of the cellular mitochondrial dehydrogenase enzyme in living cells to reduce the yellow water-soluble substrate 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) into a dark blue/purple formazan product which is insoluble in water. The amount of formazan produced is directly proportional to the cell number in a range of cells lines.At the end of 48hrs incubation, the medium in each plate containing the aqueous extract of nanophytosomesof leaves of *Momordica charantia* was added with 200µl of MTT solution and incubated for another 4hrs.The supernatant was then removed & replaced with 500µl of DMSO to dissolve the resulting MTT formazan crystals followed by mixing & measuring the absorbance at 590nm[93].

Cell viability % = OD of sample / OD of control ×100

OD: Optical Density

5.3.3. Flow Cytometric Analysis

Harvest MDA-MBcells and aliquot up to 1 x 10^6 cells/100 µL into FACS tubes. Wash the cells 2 times by adding 2 mL of PBS (or HBSS), centrifuging at 300 x g for 5 minutes, and then decanting the buffer from the pelleted cells. Resuspend cells in 100 µL of Flow Cytometry Staining Buffer. To adjust flow cytometer settings for PI, add 5 - 10 µL of PI staining solution to a control tube of otherwise unstained cells. Mix gently and incubate for 1 minute in the dark. Determine PI fluorescence (using the FL-2 or FL-3 channel) with a FACScan™ instrument. Acquire data for unstained cells and single-color positive controls. Add 5 - 10 µL of PI staining solution to each sample just prior to analysis. Set the stop count on the viable cells from a dot-plot of forward scatter versus PI[94].

RESULTS AND DISCUSSION

6. RESULTS AND DISCUSSION

Phytochemical Investigation

The results of the phytochemical study were tabulated in Table6.1. The phytochemical screening of the aqueous extract of *momordica charantia* leaves revealed the presence of steroids, flavonoids, alkaloids, sterols, phytosterols, terpenoids, tannins, proteins and amino acid, phenols, saponins. Tannins were absent in aqueous extract of *momordica charantia*.

Table6.1: Preliminary phytochemical screening of *momordica charantia* leaves extract.

S.No.	Test	Results
1.	TEST FOR ALKALOIDS	
	A. Mayer's test	+
	B. Wagner's test	+
	C. Hager's test	+
2.	TEST FOR FLAVANOIDS	
	A. NaoH test	+
	B. H_2SO_4	+
3.	TEST FOR STEROLS	
	A. Liebermann-Burchard test	+
4.	TEST FOR PHYTOSTEROLS	
	A. Salkowski test	+
5.	TEST FOR TERPENOIDS	
	A. Liebermann-Burchard test	+
6.	TEST FOR PROTEIN AND AMINO ACID	
	A. Ninhydrin(aqueous)	+
	B. Ninhydrin(acetone)s	+
	C. Biuret test	+
7.	TEST FOR ANTHRAQUINONES	
	A.Borntrager's test	+
8.	TEST FOR PHENOLS	
	A. Ferric chloride test	+
	B. Liebermann test	+
9.	TEST FOR TANNINS	
	A. Ferric chloride test	-
10.	TEST FOR SAPONINS	
	A. Foam test	+

(+)indicatespositive reaction (-)indicates negativereaction

HPTLC finger printing analysis of aqueous extract of *momordica charantia* leaves

Preliminary phytochemical investigation divulges the presence of glycosides, phenolic compounds, flavonoids, proteins, amino acids and saponins in aqueous extracts of *momordica charantia* . Hence, *momordica charantia leaves* extract containing higher alttitude of phytoconstituents which may possibly take part in reactions in effective reduction of nanophytosomess . (Martinez-Perez et al., 2014) . However, HPTLC finger print analysis also confirms the presence of MCAE (Fig. 6.1) flavonoid which has influenced the conversion of nanophytosomes due to easily oxidizable conjugated hydroxyl groups in the molecule (Terenteva, Apyari, Dmitrienko, & Zolotov, 2015).

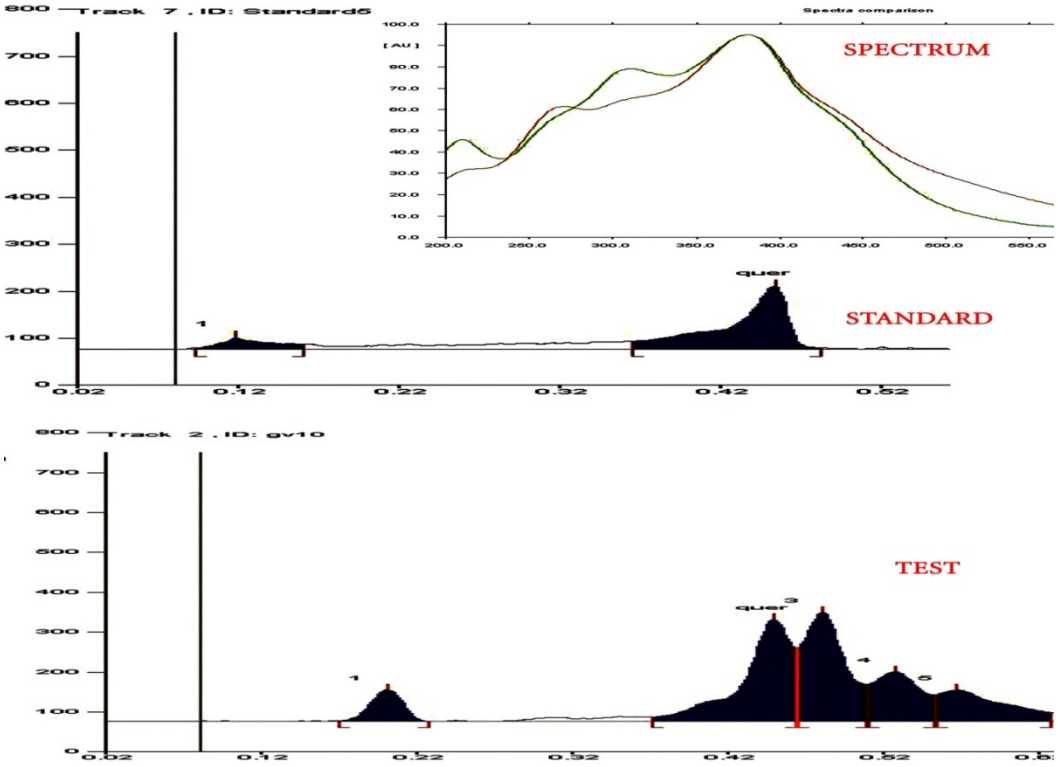


Figure6.1. HPTLC finger printing analysis of aqueous extract of *momordica charantia* leaves

Characterization of MCAE nanophytosomes

Particle size

Particle size plays an important role in the stability, availability and organoleptic properties of the solution and the particles with smaller size is desirable. Results of

particle size analysis indicated that nanophytosomes prepared with MCAE and PC possess the particle size in the average range of 584.1nm. Vesicle size tends to increase with increasing concentration of the complex. When the concentration of particles is too high, physical interaction either collision or electrostatic between vesicle is more pronoun. These interactions alter the movement of the particles and produce vesicles with a larger size. The high lipid composition in the formulation also increases the tendency for the formation of agglomerates, resulting in the bigger size of the vesicles. Polydispersity index is a measure of the heterogenecity of sizes of particles in a mixture *momordica charantia* nanophytosomes prepared show polydispersity index value of 0.4.

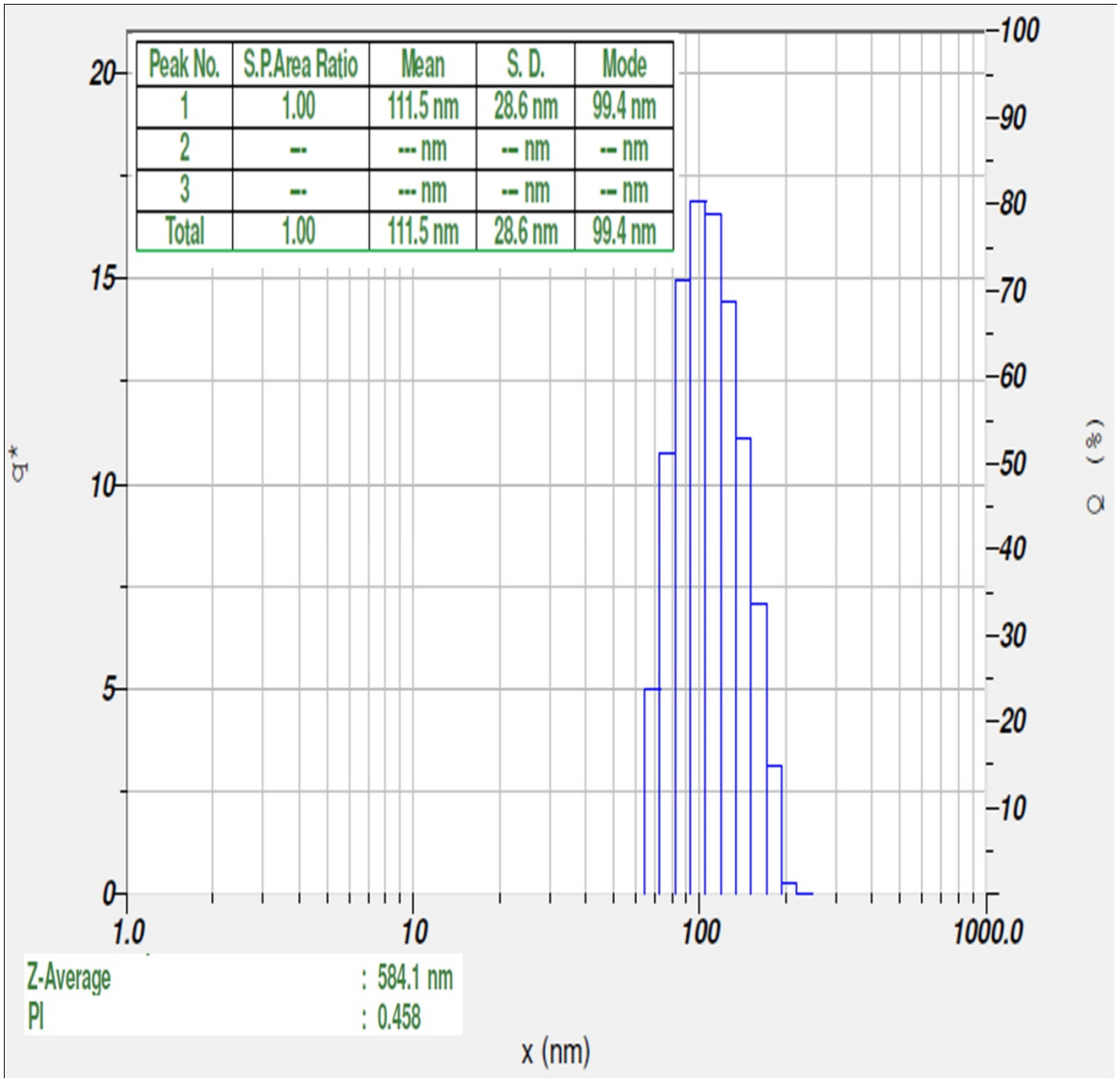


Figure 6.2 particle size of aqueous extract *momordica charantia* nanophytosomes

Zeta Potential

The Zeta potential is the electric potential in the interface or particle surface and is used to predict the stability of colloidal systems. Colloids with high absolute Zeta potential values (normally above 30 mV), regardless of their positivity or negativity, are electrically stabilized and those with low Zeta potential values are not stable and tend to coagulate or flocculate. In general, higher Zeta potential values indicate a higher and longer-term stability of the particles. Several factors such as pH, ionic strength, type and concentration of the used biopolymers are effective on the Zeta potential of the particles. The surface charge analysis results (-23.8 mV) are shown in (Figure 6.1) and point to the high physical stability of MCAE nanophytosomes.

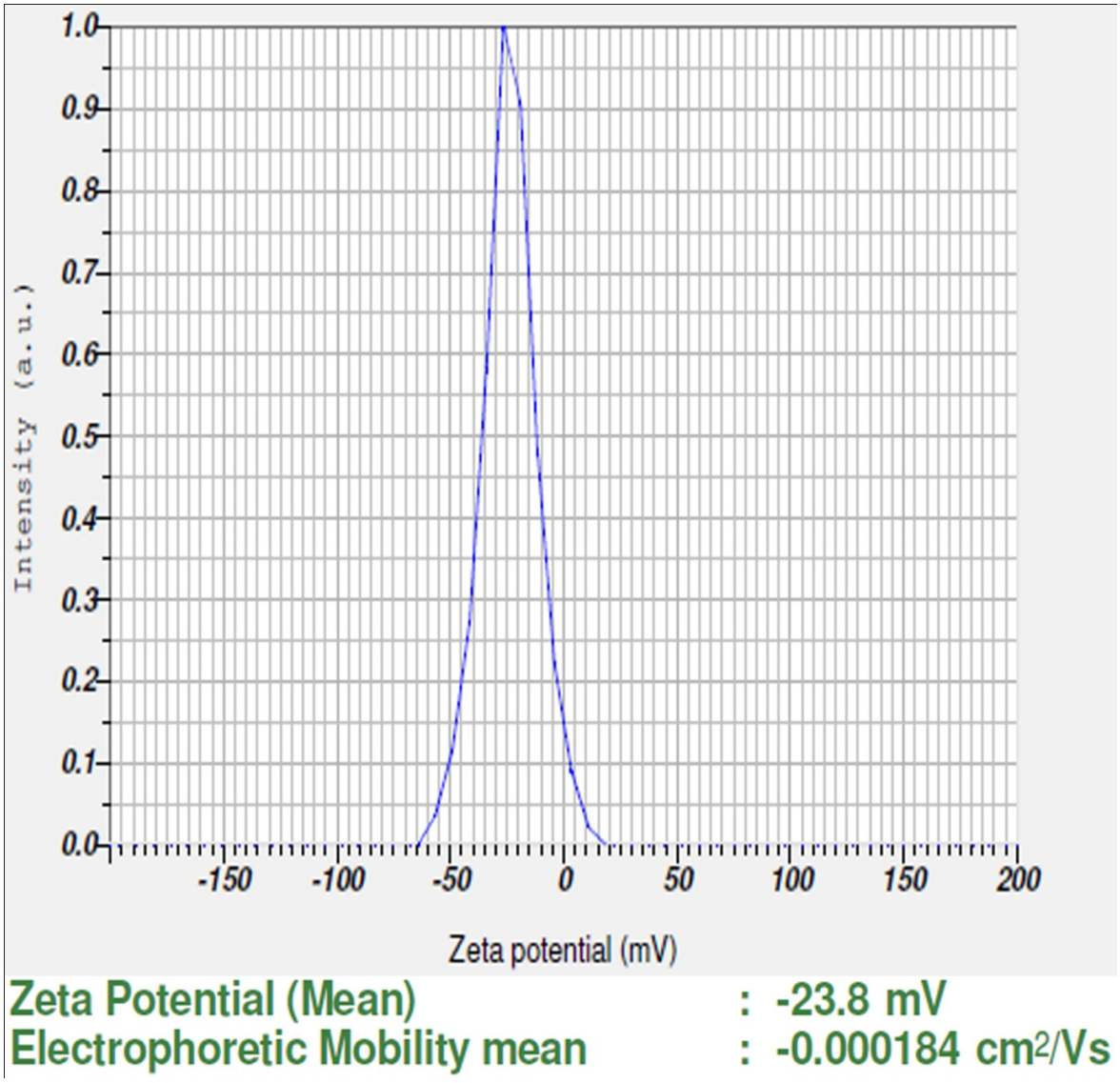


Figure6.3 Zeta Potential Distribution

Fourier Trasform-Infra Red Spectroscopy(FT-IR)Studies

Spectroscopic analysis was used in order to identify and diagnose of complex formation between PC and extract. In FTIR spectroscopy, functional groups and their numbers were identified from the frequency of radiation that absorbs infrared spectra which showed the main chemical groups in extract and PC as well as the formation of new interactions between them in the nanophytosomes preparation process. The FTIR spectroscopy of *Momordica charantia* leaf extract and excipients are shown in Figure 6.4, 6.5, 6.6. (Figure 6.4) shows that the characterstic O-H peak at 3300.20cm^{-1}, C≡N peak at 2357.01 cm^{-1}, C≡C peak at 2169.92 cm^{-1}, C=C peak at 1988.61 cm^{-1}, C=C peak at 1635.64 cm^{-1}.(Figure 6.5) shows that the C-H peak at 3020.53 cm^{-1}, C=C peak at 1988.61 cm^{-1}, C=O peak at 1734.01 cm^{-1}, C-N peak at 1215.15 cm^{-1},O-H peak at 927.76 cm^{-1}, C-H peak at 744.52 cm^{-1}, C-Br peak at 667.37 cm^{-1}. (Figure 6.6) shows that the characterstic O-H peak at 3302.13 cm^{-1}, C≡ N peak at 2360.87 cm^{-1}, C≡C peak at 2167.99cm^{-1}, C=C peak at 1990.54 cm^{-1}, C=C peak at 1635.64 cm^{-1}. Hence there is no apperence of new peaks and disapperence of existence peaks in the presence of excipients indicates the MCAE and excipients are more compatible.

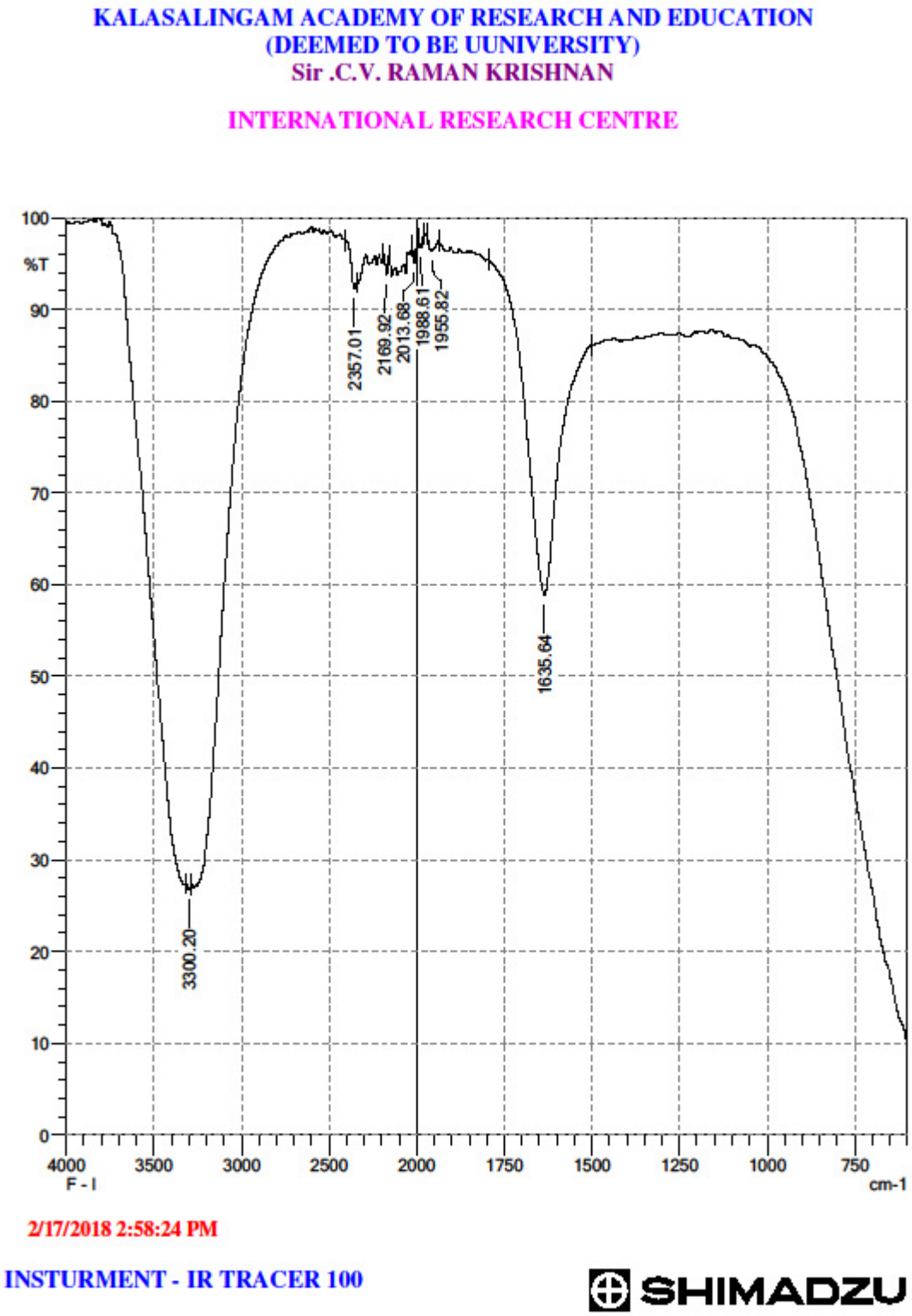


Figure 6.4. FT-IR studies of *momordica charantia* aqueous extrac.t

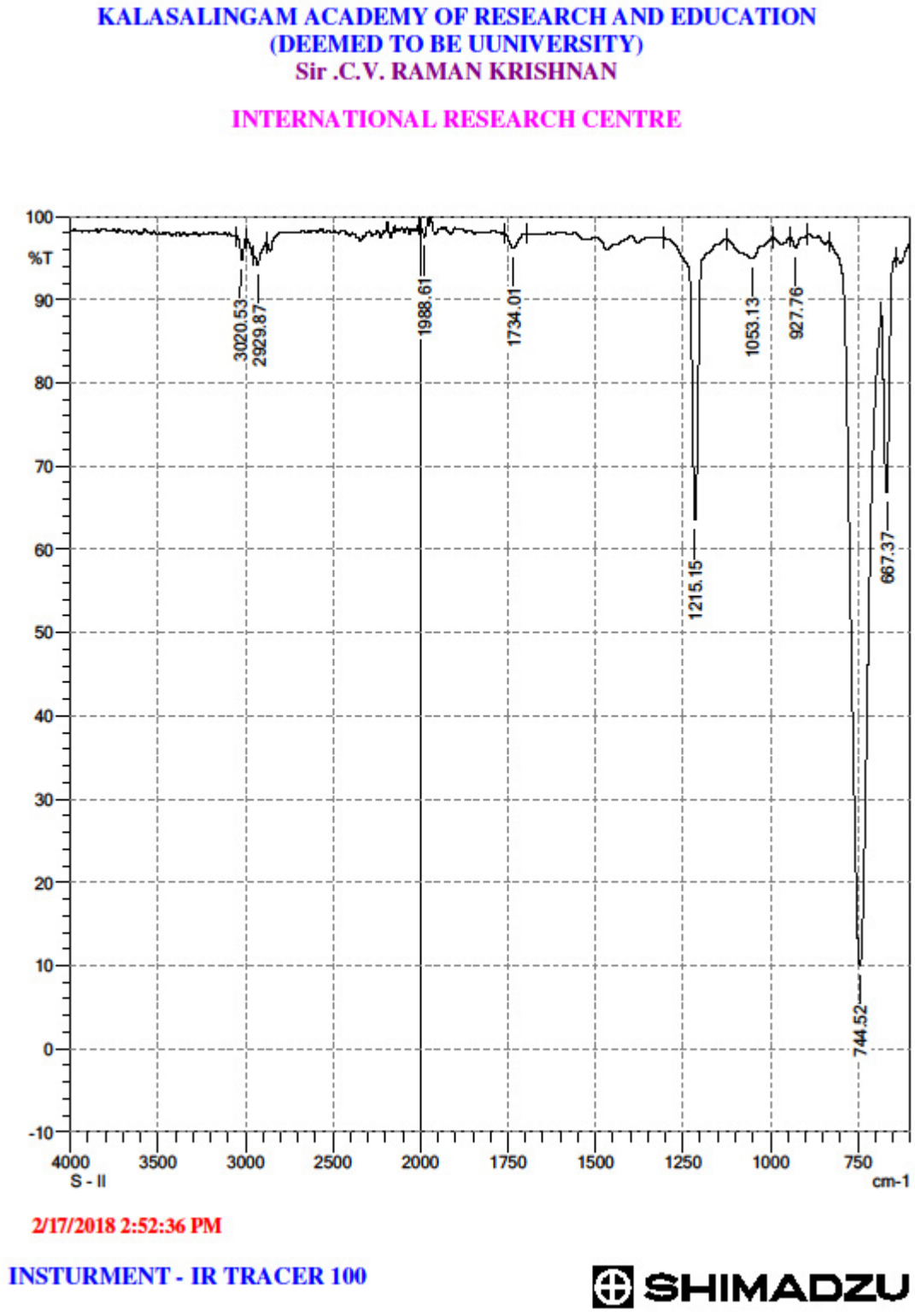


Figure. 6.5. FT-IR studies of phosphodityl-choline with cholesterol.

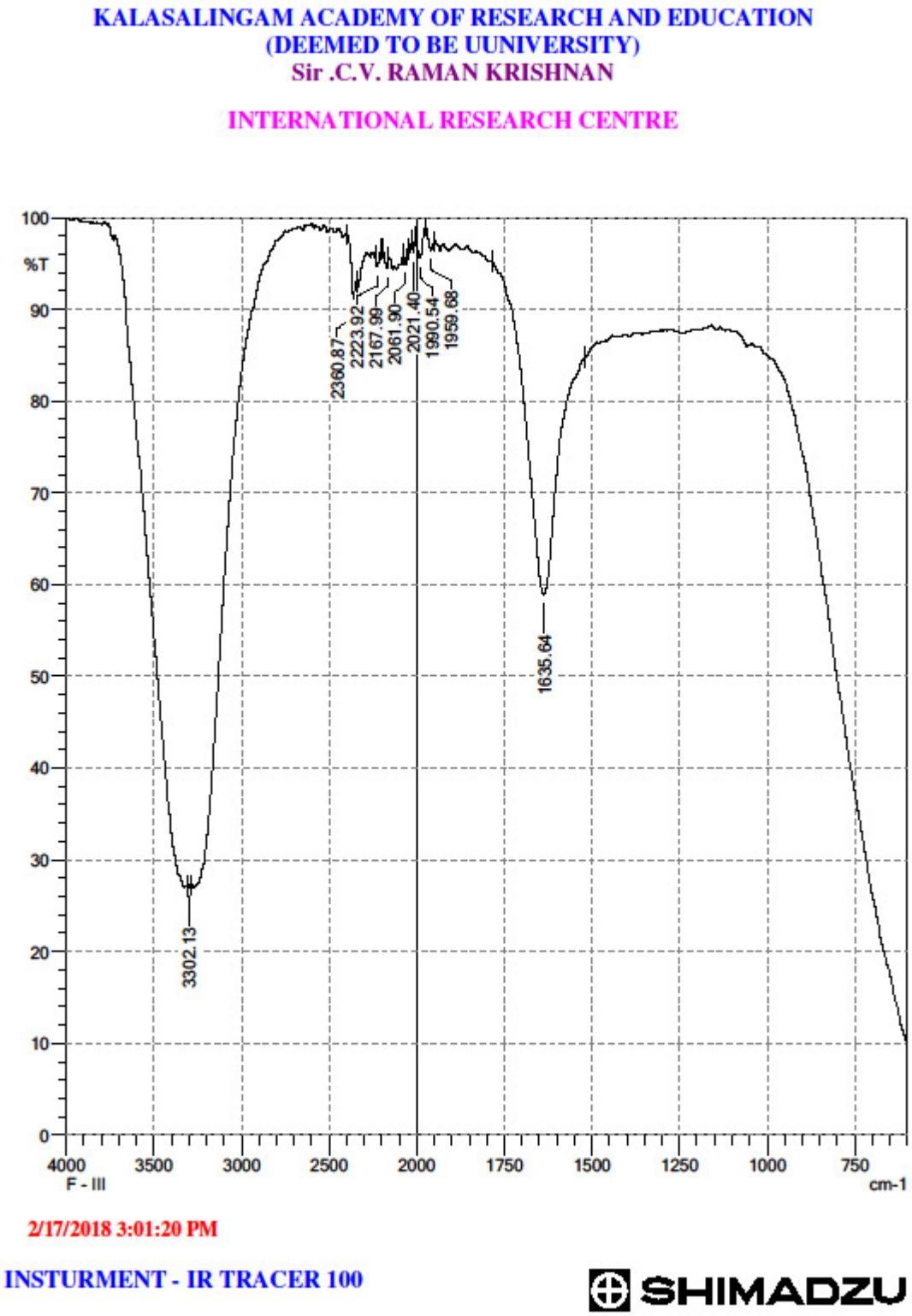


Figure.6.6. FT-IR studies of *momordica charantia* aqueous extract with excipients.

Table 6.2. Interpretation of FT-IR studies

S.No.	Functional group	Wavenumber(cm^{-1})			
		Reference	Extract	Placebo	Extraxt+placebo
1.	O-H Stretch(carboxylic acid)	3300-2500	3300.20	_	3302.13
2.	CΞN stretch(nitriles)	2260-2210	2357.01	_	2360.87
3.	CΞN stretch(alkynes)	2260-2100	2169.92	_	2169.99
4.	C=C stretch(alkenes)	1900-2000	2013.68	1988.61	2021.40
5.	C=C stretch(alkenes)	1900-2000	1988.61	_	1990.54
6.	C=C stretch(alkenes)	1900-2000	1955.82	_	1959.68
7.	C=C stretch(alkenes)	1640-1680	1635.64	_	1635.64
8.	C-H stretch(aromatic)	3100-3000	_	3020.53	_
9.	C-H stretch(alkenes)	3100-3000	_	2929.87	_
10.	C=O stretch(aldehyde, saturated aliphatic)	1720-1740	_	1734.01	_
11.	C-N stretch(aliphatic amines)	1020-1250	_	1215.15	1230.15
12.	C-N stretch(aliphatic amines)	1020-1250	_	1053.13	1072.15
13.	O-H bending(carboxylic acid)	950-910	_	927.76	928.17
14.	C-Br stretch(alkyl halides)	690-515	_	667.37	_
15.	CΞN stretch(alkynes)	2260-2100	_	_	2223.92
16.	C=C stretch(alkenes)	1900-2000	_	_	2061.90

Differential Scanning Calorimetry(DSC) Studies

Differential scanning calorimetry studied were conducted the pure MCAE, cholesterol, phosphatidylcholine. The endothermic peak of MCAE was observed at 117.7 ºC (Figure6.5) corresponding to its melting point. DSC thermogram of phosphotidyl choline and cholesterol also showed endothermic peaks at 159.4°C, respectively (Figure 6.6). DSC Thermogram of MCAE with excipients (Figure 6.7) showed endothermic peaks 132.46^{0}C. When compared the endothermic peak of extract (117.7^{0}C) with endothermic peak of extract placebo(132.46^{0}C) showed that there is no wide variation between the endothermic peaks and the difference is with in (±20^{0}C). This slight variation in the endothermic peaks may be due to the physical interaction between extract and phenol group OH.

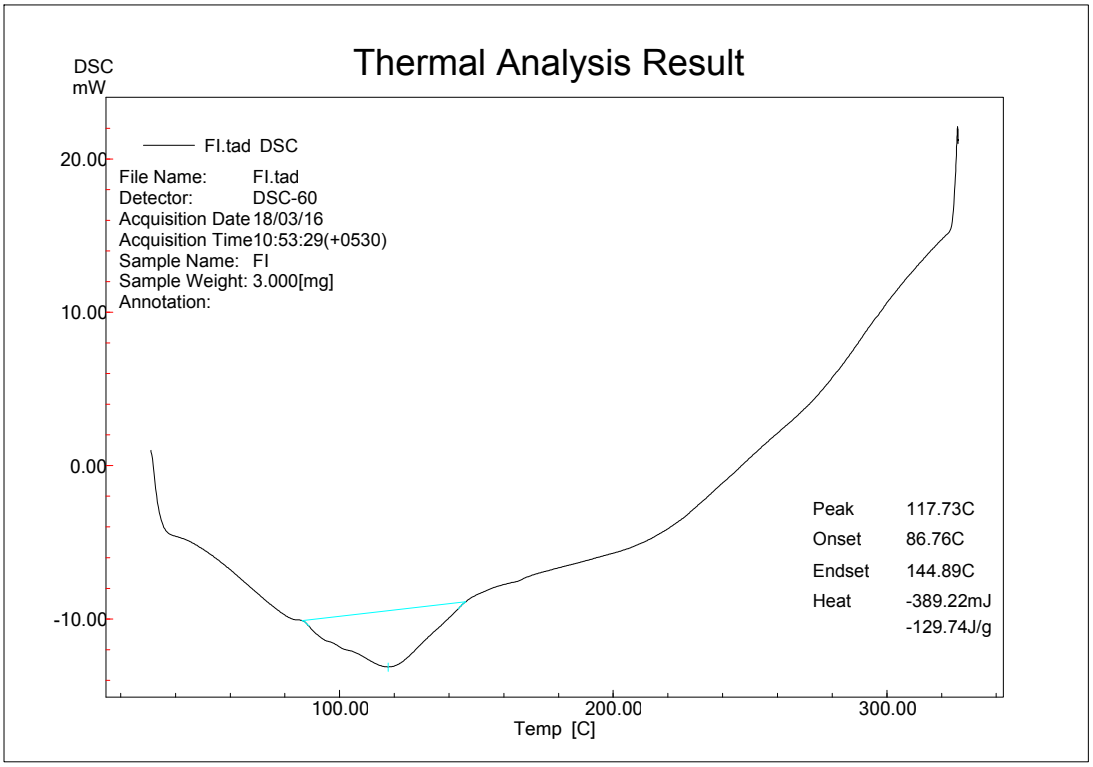


Figure. 6.7. DSC thermogram of *momordica charantia* aqueous extract.

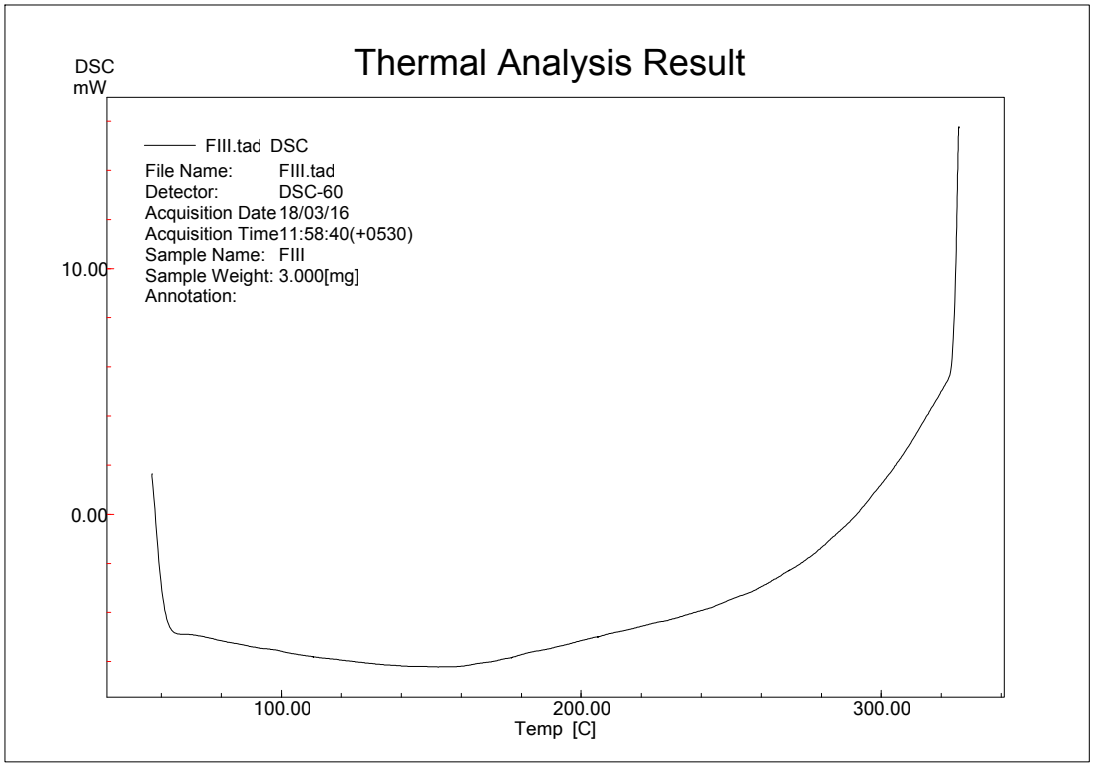


Figure.6.8. DSC thermogram of phosphotidyl choline and cholesterol.

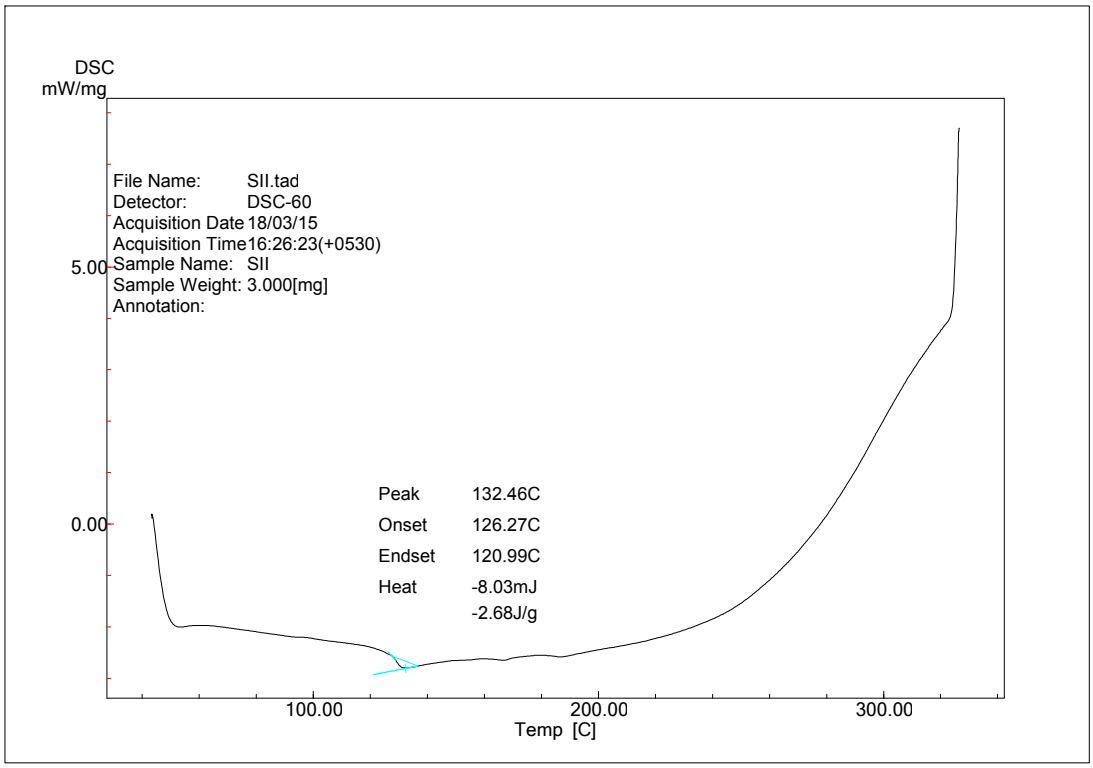


Figure 6.9. DSC thermogram of MCAE with excipients.

Scanning Electron Microscopy Studies

Scanning electron microscopy give important insight into the solid state properties and surface morphology of drug and drug complexes. SEM images(Figure 6.8) of prepared MCAE nanophytosomes ,respectively. These images showed spherical shaped MCAE nanophytosomes with a size of 100-500nm.

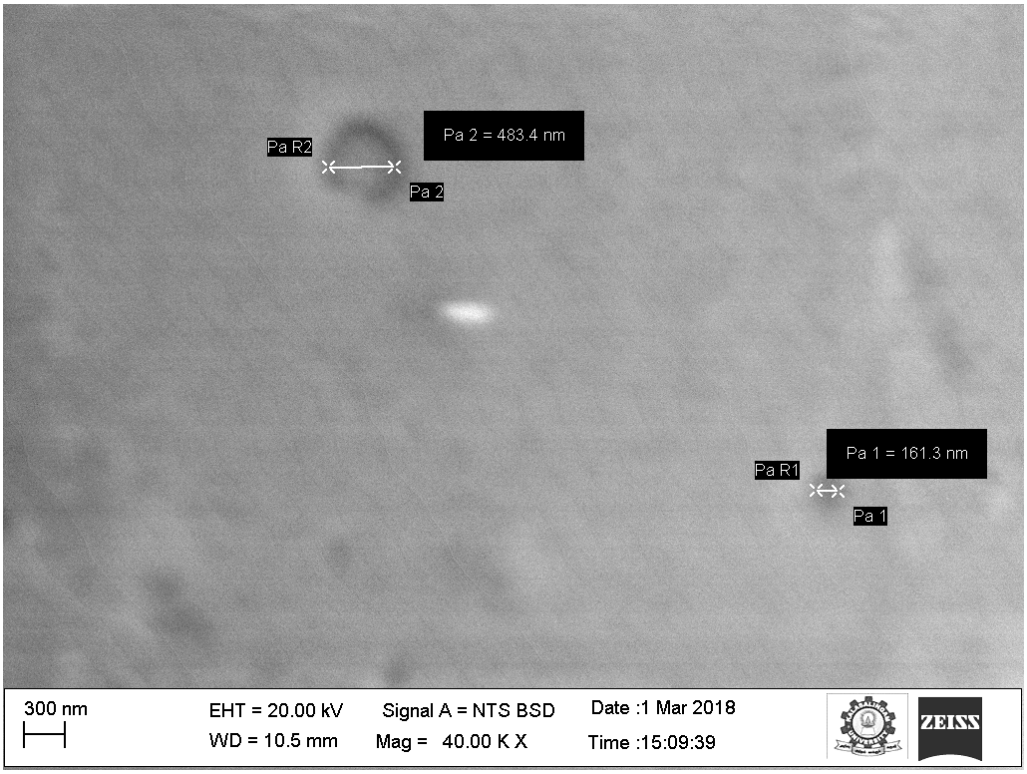


Figure6.10. SEM images for aqueous extract of *momordica charantia* nanophytosomes

IN VITRO ANTI-CANCER ACTIVITY OF *MOMORDICA CHARANTIA* PLANT EXTRACT AND NANOPHYTO SOMES

MTT assay

In this study, the cytotoxicity of aqueous extract of *momordica charantia* and nanophytosome were tested against MDA-MBhuman breast cancer cell lines. The anti-cancer property of extract and nanophytosomes was determined by MTT assay. The principle of this assay is based on the reduction of a soluble tetrazolium salt, by mitochondrial dehydrogenase activity of viable cells, into a soluble coloured formazan product that can be measured spectrophotometrically, after dissolution

with DMSO. This assay determines the integrity of mitochondria and reflects the anti-proliferative effect or cell death. The aqueous extract at 6.25, 12.5, 25, 50 and 100µg/ml (Figure6.11).

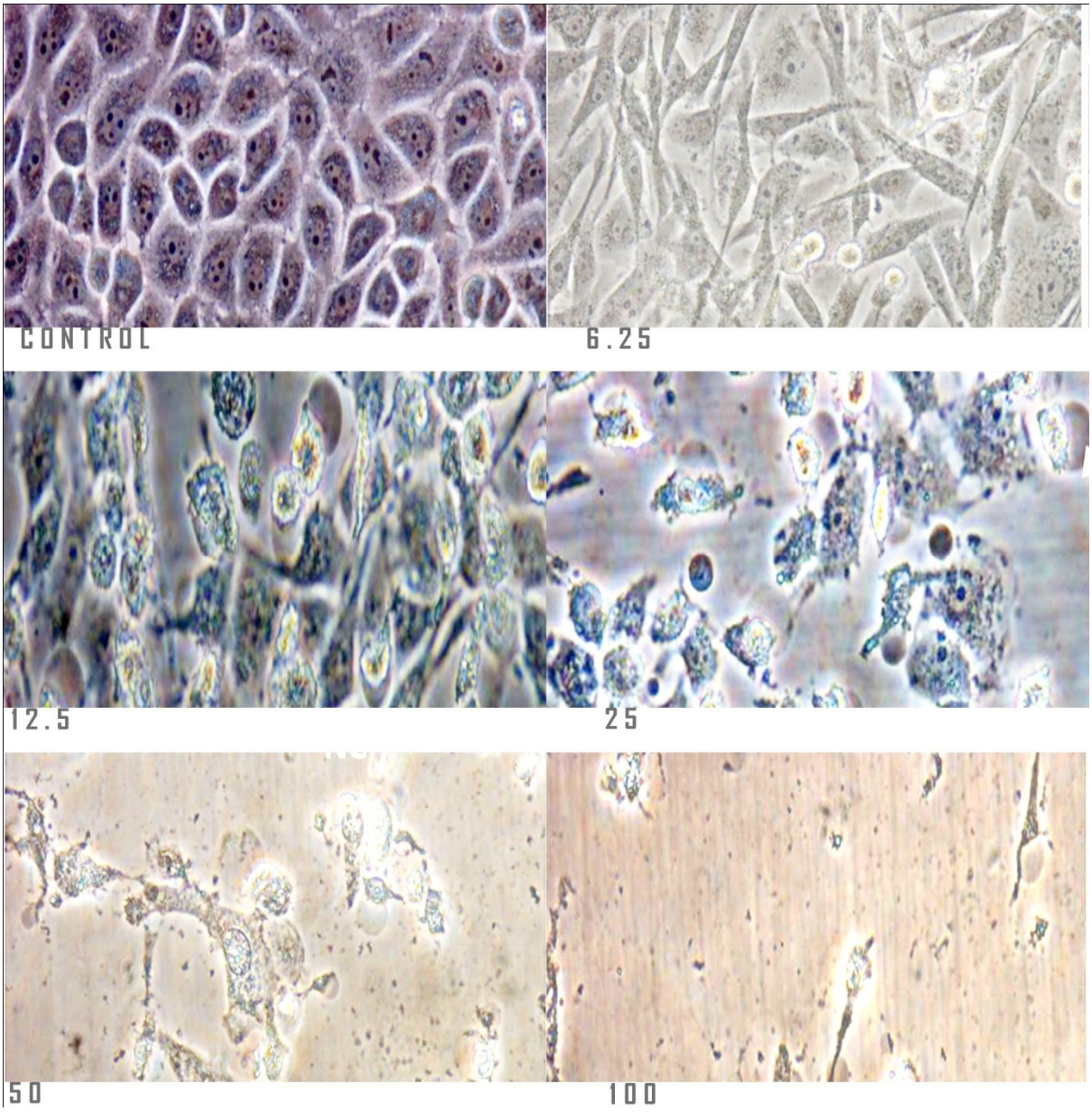


Figure6.10 MTT assay of *momordica charantia* nanophytosomes by MDA-MB breast cancer cell lines.

Table 6.3: Cell viability of MCAE Nanophytomes

S.No.	Concentration (µg/ml)	Extract (µg/ml)	Nanophytosomes (µg/ml)
1	6.25	37.8 ± o.4	32 ± 0.2
2	12.5	55 ± 0.2	41.6 ± o.4
3	25	74.5 ± 0.2	70.5 ± 0.2
4	50	84.8 ± o.4	82 ± 0.2
5	100	90 ± 0.2	96 ± 0.1

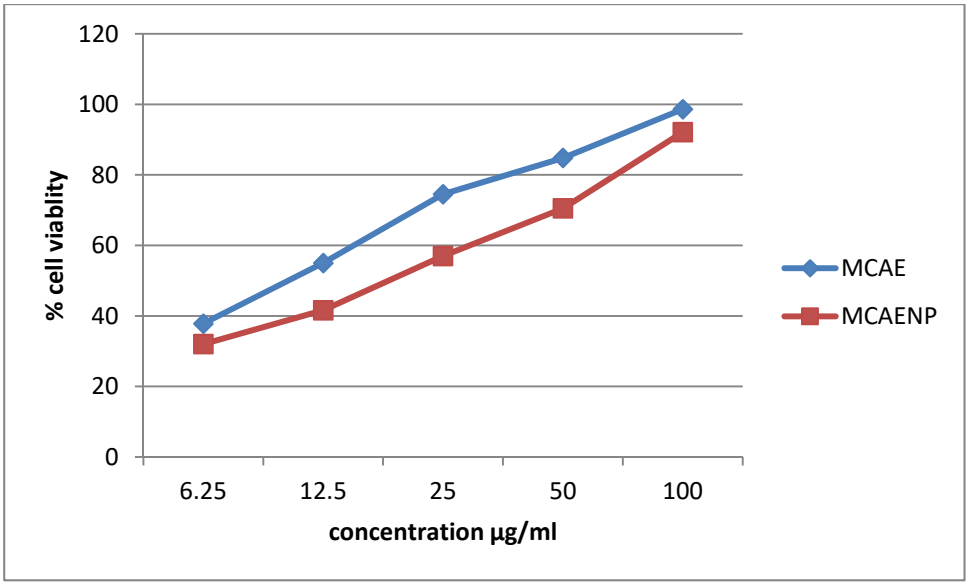


Figure 6.12.Cell viability of MCAE Nanophytosomes

CONCLUSION

7. CONCLUSION

Nanophytosomes of aqueous extract of *momordica charantia* was successfully prepared and tested for breast cancer cell lines. The aqueous extract was evaluated phyto chemical screening followed by all characterization studies. Phytochemical screening study remaining that the extract consists of flavanoids. The characterization study showed that the phytosomes are having nano size, good stability properties with round to spherical shape with smooth surfaces. The MTT assay, extract and nanophytosomes showed that nanophytosomes are having more anti cancer activity. When compared with aqueous extract against MDA-MBbreast cancer cell lines. Hence, the prepared nanophytosomes showed excellent in vitro anti cancer activity. This work can be further extended to clinical trials.

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Review Article

A REVIEW ON PHYTOSOMES, IMPORTANCE AND ITS APPLICATIONS

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ABSTRACT

Phytosomes are said to be natural extracts contains plant constituents which are bound in phospholipids mainly phosphotidylcholine by producing a lipid stable molecular complexes. This will become better formulation with high grade of stability to attain peak pharmacokinetic and pharmacodynamic profiles. Such degree of freedom will pave to chart out many therapeutic interventions towards the treatment of most of diseases. Hence, The authors has taken a lead to emphasize the importance of phytosomes, its preparations and characterization and also current scenario towards phytosomal technology in detailed way.

Key words: Nanotechnology; Plant products; Nanophytosomes.

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INTRODUCTION

Phytosomes are said to be containing natural herbal formulations. Most of the Plants are having medicinal properties due to the presence of many active constituents which are mainly the secondary metabolites like flavonoids, terpinoids, tannins, glycosides, alkaloids etc. The active constituents present in the plants are mostly hydrophilic in nature. The therapeutic efficacy of herbal extracts are quickly destroyed by the enzymes present in the intestinal gut. Hence, advanced researches are done for the specific site delivery of these plants derived products (Middleton and Kandaswami,1994).The term "phyto" means plant and "some" means cell like (Mukherjee and Wahile,2006). It is also called as herbosomes. This is an advanced methodology, where extract of the plant or the hydrophilic phytoconstituents are mixed with phospholipids to produce more lipid stable molecular complexes, thereby it enhances the absorption and bioavailability of phytoconstituents (Manach *et al.*, 2004; Mascarell, 1993). Phospholipids are naturally used as an aid for digestion and act as carriers for both fat soluble and water soluble nutrients (Shivan and Kinjal, 2010). Phytosomes can easily cross the cell membranes and also stratum corneum layer of the skin (Bombardelli *et al*., 1989; Bombardelli, 1991; Bombardelli and Spelta,

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